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Article

Animal Welfare Policy: Implementation in the Context of Wildlife Research—Policy Review and Discussion of Fundamental Issues

Ellen Paul, Robert S. Sikes, Steven J. Beaupre, and John C. Wingfield

Ellen Paul, JD, MS, is Executive Director of the Ornithological Council. Robert S. Sikes, PhD, is Professor of Biology at the University of Arkansas at Little Rock, Chair of the Animal Care and Use Committee, and President-elect of the American Society of Mammalogists. Steven J. Beaupre, PhD, is Professor and Chair in the Department of Biology at the University of Arkansas, Fayetteville, and Past President of the American Society of Ichthyologists and Herpetologists. John C. Wingfield, PhD is Distinguished Professor of Neurobiology, Physiology, and Behavior at the University of California, Davis, and Past Director of the Division of Integrative Organismal Systems of the BIO division of the National Science Foundation

Address correspondence and reprint requests to Ellen Paul, Executive Director, The Ornithological Council, 6512 E. Halbert Rd., Bethesda, MD 20817. or email ellen.paul@verizon.net.

Abstract

The use of vertebrate animals in research and education in the United States is subject to a number of regulations, policies, and guidelines under the immediate oversight of Institutional Animal Care and Use Committees (IACUCs), which are charged with ensuring the ethical and appropriate use of the animal subjects. In almost all instances, this regulatory and oversight landscape of animal use has been developed around domesticated animals in biomedical research environments. When the research activities involve wild species, especially in their natural habitat rather than a laboratory, oversight personnel and investigators alike struggle with determining what constitutes ethical and appropriate activities. These difficulties stem from fundamental differences in biology between wild and domesticated animals and from the differences in research objectives and methods in wildlife compared with biomedical research. Here we discuss the various policies, regulations, and guidance documents for animal use in the context of wildlife research. We compare the expectations of the various oversight agencies and how these expectations are met when working with wild vertebrates. We make recommendations for how IACUCs can use available resources to ensure that activities involving wild species are conducted in compliance with existing regulations and policies and in ways that are biologically appropriate for these nondomesticated species.

Key words: animals; animal care committee; animal experimentation; animal oversight; animal welfare; Institutional Animal Care and Use Committee (IACUC); wild; wildlife

Introduction

In the early 1950s, leaders of the biomedical research community organized a committee to address animal welfare issues. Two years later, the National Academy of Science established its own committee on animal resources "for the purpose of recommending a long-term procurement and supply mechanism of animals for biologic, medical, and agricultural research" (Wolfle 1999, 44). That committee's work resulted in the formation of the Institute of Animal Resources—now the Institute of Laboratory Animal Research (ILAR). These two organizations

then collaborated in writing the 1963 *Guide for Laboratory Animal Facilities and Care*, which was published by the US Department of Health Education and Welfare. The document later came to be called *Guide for the Care and Use of Laboratory Animals* (commonly referred to as the "ILAR Guide" or just "the *Guide*"). Then, and through seven revisions, there was no evidence that wildlife biologists were ever consulted, included on the committee of experts who revised the text, or included among the reviewers. Consequently, it is not surprising that the *Guide* never addressed wildlife biology until recent revisions, and then in a very cursory and broad manner (Sikes et al. 2012). The *Guide*, which is the standard mandated by the 1986 Public Health Service (PHS) Policy, has little information relevant to wildlife research beyond general principles.

The National Science Foundation (NSF), which funds most federally supported wildlife research, recognized that there were no accepted humane policies pertaining to wildlife research and, therefore, Institutional Animal Care and Use Committees (IACUCs) had no biologically appropriate guidance for reviewing protocols for wildlife research (Orlans 1988). To address this situation, the NSF in 1986 urged the presidents of taxon-oriented scientific societies to develop guidelines for the appropriate handling of their taxa. Funding from the NSF facilitated these efforts (Orlans 1988), and taxon-based guidelines for mammals, birds, reptiles and amphibians, and fishes were published in 1987 and 1988. Each of these documents has been revised several times, and each revision has entailed rigorous peer review and accretion in the number of citations (Sikes et al. 2012).

The National Science Foundation in 2013 gave formal recognition to these taxon-specific guidelines by way of a revision to the Grant Proposal Guide (NSF 2014):

> Proposals Involving Vertebrate Animals
> a. Any project proposing use of vertebrate animals for research or education shall comply with the Animal Welfare Act [7 U.S.C. 2131 et seq.] and the regulations promulgated thereunder by the Secretary of Agriculture [9 CFR 1.1-4.11] pertaining to the humane care, handling, and treatment of vertebrate animals held or used for research, teaching or other activities supported by Federal awards. In accordance with these requirements, proposed projects involving use of any vertebrate animal for research or education must be approved by the submitting organization's Institutional Animal Care and Use Committee (IACUC) before an award can be made. For this approval to be accepted by NSF, the organization must have a current Public Health Service (PHS) Approved Assurance.
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> In the case of research involving the study of wildlife in the field or in the lab, for the provision in the PHS Assurance for Institutional Commitment (Section II) that requires the organization to establish and maintain a program for activities involving animals in accordance with the *Guide for the Care and Use of Laboratory Animals* (*Guide*), the organization has established and will maintain a program for activities involving animals according to the *Guide*. The organization will follow recommendations specified in the *Guide* for details involving laboratory animals, and taxon-specific guidelines approved by the American Society of Ichthyologists and Herpetologists, the American Society of Mammalogists, and the Ornithological Council, as is appropriate for the taxon to be studied.

The taxon-based scientific organizations have engaged in substantial outreach to IACUCs, institutional officials, and researchers to make them aware of this change, have encouraged the use of taxon-specific guidelines, and have developed a model wildlife protocol (provided in full in Supplementary Appendix C). However, it is apparent that there is still uncertainty and confusion on the part of researchers and IACUC members about which policies and regulations apply to them, what those policies actually require, and how to apply available guidance in the context of wildlife research in the field and in the laboratory, where the species, individuals, and conditions differ greatly from those typical in biomedical research.

This document is intended to guide researchers and IACUCs by (1) providing the actual text of the relevant policies; (2) explaining how to determine which policies apply to a specific project; (3) attempting to "fill gaps" between policies and guidance written for biomedical research and the realities of wildlife biology, consistent with the "spirit of the law"; and (4) explaining what is required of researchers and oversight personnel when writing or reviewing protocols that include methods not addressed by or seemingly at odds with the relevant policies.

Every effort has been made by the authors, in consultation with the National Institutes of Health (NIH) Office of Laboratory Animal Welfare (OLAW) and the US Department of Agriculture (USDA) Animal and Plant Health Service Animal Care program, to express correct interpretations of federal policy within the context of the specific issues being discussed. However, in this highly nuanced field, readers are cautioned not to apply these interpretations out of context or to extend them outside the context of wildlife research. Readers are therefore advised to refer to source documents and engage in direct consultations with the federal agencies.

The differences between biomedical research and wildlife research are vast:

- Biomedical researchers use animals as model organisms to gain an understanding of specific diseases or conditions, generally for the benefit of humans. In contrast, the actual wild animals, their biology, behavior, and environment are the focus of wildlife research. These animals are typically studied for their own sake and not as models for other organisms, although in rare instances, particularly in the case of highly endangered species, one species may be studied as a surrogate for another. Even in these rare instances, however, the focus remains on the basic biology of wild taxa.
- Almost all biomedical research is performed in laboratory settings where investigators have complete control over the environment, number, gender, and even genetic traits of the animals studied. Wildlife research, on the other hand, usually takes place in the natural habitat of the animals being studied —an environment that can never be fully controlled and where conditions and resources typical of laboratory environments are absent. Even when wildlife are studied in captivity, the husbandry of wild animals differs substantially from that of domesticated species bred specifically for research.
- It is estimated that purpose-bred rats and mice comprise 85–95% of all animals used in biomedical research. By comparison, wildlife research can entail any of more than 60,000 species of vertebrates. Wildlife studies can be conducted in the field, in the laboratory, or in seminatural enclosures. They may require only observation of animals in the wild or they might involve capture and manipulation of individuals. The environmental conditions in field studies are dictated by nature and are not under the control of investigators except, to a limited extent, for minor or temporary manipulations.

As a consequence of these differences, application of regulations, policies, and standards written for biomedical studies to wildlife research is problematic at best. From basic principles to practical matters, the fundamental differences in wild versus domesticated animal systems will generally result in very different outcomes. So, for instance, the concept of replacing animals with less sentient taxa or with nonanimal models will rarely apply to wildlife studies. Occupational safety and health issues are more

varied and complex and require a different knowledge base. The role of the attending veterinarian is altered because veterinarians rarely accompany researchers to field sites, especially not on a routine basis to provide veterinary care.

This guidance addresses basic issues associated with protocol review, training, postapproval monitoring, and annual reporting. We address one specific methodological topic—euthanasia—because it generates many questions, particularly in the light of the 2013 revisions to the American Veterinary Medical Association Guidelines for the Euthanasia of Animals (AVMA 2013). We also address Animal Welfare Act (AWA) implementation in the context of the study of wildlife by federal and state agencies.

It is our hope that this policy review and discussion, together with other resources listed in the Supplementary Appendices A–C, will make it possible for funding agencies, members of Institutional Animal Care and Use Committees, veterinary medical officers and inspectors of Animal and Plant Health Inspection Service (APHIS) Animal Care, members of the AAALAC International Council on Accreditation, institutional officials, and researchers to ensure that wildlife research is compliant with the relevant policies yet realistic given the conditions and limitations inherent in wildlife research.

Methods and Sources

We begin with a review of the existing statutes, regulations, and policies to determine what, if anything, each authority states or implies for each question:

- AWA [7 U.S.C. 2131–2159] and AWA Regulations (9 CFR 1.1–3.142)
- Health Research Extension Act of 1985 [42 U.S.C. §289 (d)]
- PHS Policy (NIH-OLAW 2015b)
- US Government Principles (USGP) for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training (IRAC 1985)
- *Guide for the Care and Use of Laboratory Animals* ("the *Guide*"); (NRC 2011)

We use the abbreviation AWA to refer to both the statute and the implementing regulations but give the actual citation to the authority cited; in most cases, the regulations are more detailed than the statute, so most references are to the regulations. A citation to the CFR (Code of Federal Regulations) is a citation to the regulations. A citation to the USC (United States Code) is a citation to the statute.

We also made liberal use of information published on the OLAW website, including the OLAW frequently asked questions (FAQs) (NIH-OLAW 2015a). Note that in the FAQs, OLAW states:

> OLAW develops and monitors, as well as exercises compliance oversight relative to the Public Health Service Policy on Humane Care and Use of Laboratory Animals (the "PHS Policy"). One of OLAW's primary functions is to advise awarding units and awardee institutions concerning the implementation of the PHS Policy. OLAW often provides this advice by responding to policy-related questions submitted by such units and institutions. The following FAQs provide guidance that represents OLAW's current thinking on these topics. This guidance is based on OLAW's experience with the subject matter and draws on best practices followed by the biomedical community regarding the use of research animals. Unless specific statutory or regulatory requirements are cited, the FAQs should be viewed as recommendations in that an institution may use an alternative approach if the approach satisfies the requirements of the PHS Policy.

We then provide analyses intended to guide the user in applying these regulations, policies, guidelines, and standards (all written for biomedical science) to wildlife research. We attempt to fill the many gaps left by the existing policies and guidance with:

- scientific knowledge derived from more than 100 years of published, peer-reviewed research;
- the peer-reviewed compendia of this research published by our scientific societies (*Guidelines to the Use of Wild Birds in Research* [Fair *et al.* 2010], *Guidelines of the American Society of Mammalogists for the Use of Wild Mammals in Research* [Sikes *et al.* 2011], *Guidelines for the Use of Live Amphibians and Reptiles in Field and Laboratory Research,* [Beaupre *et al.* 2004] and *Guidelines for the Use of Fishes in Research* [Use of Fishes in Research Committee 2014]); and
- insights from extensive discussions with hundreds of highly experienced wildlife biologists and IACUC members.

Wherever possible, this guidance gives straightforward yes-or-no answers, but the reality is that, in most instances, uncertainty characterizes the implementation of animal welfare laws in the context of wildlife research.

Abbreviations and Acronyms

AAALAC, AAALAC International
AFS, American Fisheries Society
APHIS, Animal and Plant Health Inspection Service
ASIH, American Society of Ichthyologists and Herpetologists
ASM, American Society of Mammalogists
AVMA, American Veterinary Medical Association
AVMA Guidelines, AVMA Guidelines for the Euthanasia of Animals (2013)
AWA, Animal Welfare Act
AZA, Association of Zoos and Aquariums
CFR, Code of Federal Regulations
CITI, Collaborative Institutional Training Initiative
Guide ("the *Guide*"), Guide for the Care and Use of Laboratory Animals, published by the Institute for Laboratory Animal Research, National Research Council of the National Academies (8th ed, 2011)
IACUC, Institutional Animal Care and Use Committee
IATA, International Air Transport Association
NIH, National Institutes of Health
NSF, National Science Foundation
OC, Ornithological Council
OLAW, Office of Laboratory Animal Welfare
PHS, Public Health Service
USC, United States Code;
USDA, US Department of Agriculture
USGP, US Government Principles

Glossary and Usage Guide

The definition and usage of key terms varies between agencies and policies. The usage of key terms in this document differs from policy in some cases; it is more reflective of the actual usage by wildlife biologists. For this reason, the authors provide both official definitions of key terms and explain usage in the discussion sections of this document. The usage is that of the authors and does not imply acceptance by any federal agency.

Animal

AWA

"Animal means any live or dead dog, cat, nonhuman primate, guinea pig, hamster, rabbit, or any other warm-blooded animal,

which is being used, or is intended for use for research, teaching, testing, experimentation, or exhibition purposes, or as a pet. This term excludes birds, rats of the genus *Rattus,* and mice of the genus *Mus,* bred for use in research; horses not used for research purposes; and other farm animals, such as, but not limited to, livestock or poultry used or intended for use as food or fiber, or livestock or poultry used or intended for use for improving animal nutrition, breeding, management, or production efficiency, or for improving the quality of food or fiber. With respect to a dog, the term means all dogs, including those used for hunting, security, or breeding purposes" (9 CFR 1.1).

PHS Policy

"Animal—Any live, vertebrate animal used or intended for use in research, research training, experimentation, or biological testing or for related purposes" (NIH-OLAW 2015b).

As Used in Discussion Herein

Animal is any live vertebrate animal used or intended for use in research, research training, experimentation, or biological testing or for related purposes. Following Sikes and Paul (2013, 15):

> The term "use," as in animals "used" in research, is laden with connotations and values that differ greatly between biomedical and wildlife research. Rather than serving merely as a utilitarian model, wild animals are the focus and often the beneficiaries of wildlife research. Unfortunately, "use" as the verb is well-established in regulatory language and is found throughout documents familiar to IACUCs. Even the guidelines published by professional taxon-based societies have defaulted to this language, presumably to be consistent with the expectations of IACUCs and regulatory personnel. Prior concessions to common usage notwithstanding, throughout this article we will eschew the term "use" as a verb when speaking of non-biomedical research.

Euthanasia

AWA

"Euthanasia means the humane destruction of an animal accomplished by a method that produces rapid unconsciousness and subsequent death without evidence of pain or distress, or a method that utilizes anesthesia produced by an agent that causes painless loss of consciousness and subsequent death." (9 CFR 1.1).

PHS Policy

"Methods of euthanasia used will be consistent with the recommendations of the American Veterinary Medical Association (AVMA) Panel on Euthanasia, unless a deviation is justified for scientific reasons in writing by the investigator." (NIH-OLAW 2015b).

As Used in Discussion Herein

Euthanasia is a humane method of terminating life.

Field Study

AWA

"Field study means a study conducted on free-living wild animals in their natural habitat. However, this term excludes any study that involves an invasive procedure, harms, or materially alters the behavior of an animal under study" (9 CFR 1.1).

PHS Policy

PHS Policy does not provide a definition.

As Used in Discussion Herein

The usage in the discussions is consistent with the AWA definition. See discussion for challenges in application of the AWA definition.

Principal Investigator

AWA

"Principal investigator means an employee of a research facility, or other person associated with a research facility, responsible for a proposal to conduct research and for the design and implementation of research involving animals" (9 CFR 1.1).

PHS Policy

PHS Policy does not provide a definition.

As Used in Discussion Herein

The usage in the discussions recognizes that the principal investigator (PI) is responsible for submission of the protocol and for oversight of all project staff, including compliance with the approved protocol. The discussions use the broader term "researcher" to include everyone who works on the study. In terms of methods, occupational safety, and all other aspects of the study, we do not differentiate among PIs, co-investigators, field or lab technicians, students, and volunteers.

Study Area, Animal Facility, Study Site, Field Site

AWA

"Study area means any building room, area, enclosure, or other containment outside of a core facility or centrally designated or managed area in which animals are housed for more than 12 hours" (9 CFR 1.1).

PHS Policy

Animal Facility—Any and all buildings, rooms, areas, enclosures, or vehicles, including satellite facilities, used for animal confinement, transport, maintenance, breeding, or experiments inclusive of surgical manipulation. A satellite facility is any containment outside of a core facility or centrally designated or managed area in which animals are housed for more than 24 hours (NIH-OLAW 2015b).

As Used in Discussion Herein

The terms "study site" and "field site" describe a place where animals are studied; in the context of this guidance, study sites and field sites are generally located in the natural habitat of the animals and may be fixed or ephemeral. If animals are removed from their natural habitat, the study site would include both places where animals are housed and places where the actual research occurs.

Thoracic Compression/Cardiac Compression

AVMA Guidelines for the Euthanasia of Animals

The term used by the AVMA is thoracic (cardiopulmonary, cardiac) compression. It is not described or defined (AVMA 2013).

As Used in Discussion Herein

Recent research (J Paul-Murphy, unpublished data) confirms that as to birds, the appropriate term is cardiac compression. Therefore, when used in this document, distinction is made as to birds by use of the term cardiac compression.

Which Policies Apply?

If an institution receives PHS funding, it must provide a written Animal Welfare Assurance (Assurance) acceptable to the PHS, setting forth compliance with PHS Policy. Both PHS Policy and the PHS Sample Assurance document expressly require that institutions establish and maintain a program for activities involving vertebrate animals according to the *Guide for the Care and Use of Laboratory Animals* (NRC 2011) for all PHS-supported activities. Although other federal funding agencies adhere to the USGP (IRAC 1985), they have no formal programs for implementing the AWA as to extramural research (i.e., grants and contracts) and do not require Assurance documents from their grantees or contractors. For instance, the USDA's National Institute of Food and Agriculture Federal Assistance Policy Guide requires only IACUC approval.

The NSF has a similar requirement; the Grant Proposal Guide (NSF 2014) states: " [P]roposed projects involving use of any vertebrate animal for research or education must be approved by the submitting organization's Institutional Animal Care and Use Committee (IACUC) before an award can be made. For this approval to be accepted by NSF, the organization must have a current Public Health Service (PHS) Approved Assurance."

However, the NSF Grant Proposal Guide (NSF 2014) has, since 2013, specified the use of the taxon-specific guides for the study of wildlife:

> In the case of research involving the study of wildlife in the field or in the laboratory, for the provision in the PHS Assurance for Institutional Commitment (Section II) that requires the organization to establish and maintain a program for activities involving animals in accordance with the *Guide for the Care and Use of Laboratory Animals* (Guide), the organization has established and will maintain a program for activities involving animals according to the Guide. The organization will follow recommendations specified in the Guide for details involving laboratory animals, and taxon-specific guidelines approved by the American Society of Ichthyologists and Herpetologists, the American Society of Mammalogists, and the Ornithological Council, as is appropriate for the taxon to be studied.

Although a PHS Assurance is strictly required only for those projects supported by PHS funds, institutions have the option of altering the default language of the PHS sample assurance document so that the PHS Assurance covers all animal activities. When the PHS Assurance is written so as to include all research with vertebrate animals, then PHS policies and compliance with the *Guide* is expected for all activities.

The PHS FAQ A.1 (NIH-OLAW 2015a) states:

> There are many valid reasons for institutions to perform program oversight institution-wide using uniform and consistent standards for animal care and use. Likewise, it is generally impractical to separate activities based on the source of funding. Institutions must implement the PHS Policy for all PHS supported activities involving animals, and must ensure that any standards that might not be consistent with PHS Policy do not affect or pose risks to PHS supported activities.
>
> It is permissible for institutions to delineate animal areas that are programmatically and functionally separate and that do not support PHS animal activities such as a herd of beef cattle used for food production or a stable of riding horses. The Assurance should explicitly reflect the exclusion of any specific area or activity.

In August 2015, OLAW and the NSF entered into a Memorandum of Understanding (MOU) (NIH 2015) (effective October 1, 2015) whereby OLAW would ensure compliance with the PHS Policy on Humane Care and Use of Laboratory Animals (PHS Policy) for all NSF grants and cooperative agreements involving research with live vertebrate animals.

Under this MOU, OLAW is to do the following:

1. Negotiate, review and approve Assurances in support of NSF grants, and cooperative agreements.
2. Distribute lists of approved Assurances on the OLAW website.
3. Advise and educate on implementation of the PHS Policy.
4. Provide guidance on interpretation of the PHS Policy.
5. Evaluate allegations of noncompliance with the PHS Policy.
6. Authorize waivers to the PHS Policy.
7. Conduct site visits to selected institutions.

In addition, at the request of NSF, OLAW will negotiate Assurances for NSF-affiliated institutions that do not receive direct PHS support for activities involving live vertebrate animals.

The authors have been assured by NSF officials that, this MOU notwithstanding, the terms of the NSF Grant Procedure Guide, which direct that the taxon-specific guidelines be followed for wildlife research, will be retained. It is not clear, as of this writing, how the NSF/OLAW MOU will address this apparent conflict.

Understanding the scope of applicability of an institution's Assurance is essential. Under PHS Policy (NIH-OLAW 2015b), all activities funded by the PHS must be conducted in a manner that is consistent with a single standard, the *Guide for the Care and Use of Laboratory Animals* (NRC 2011). With the MOU between OLAW and the NSF (NIH 2015), Assurances will also cover these non-PHS-funded activities. If the Assurance is written so as to specify that PHS Policy applies only to PHS-funded research, wildlife researchers and their institutions who receive NSF and other non-PHS funding for wildlife research will also be able to comply with the NSF Grant Proposal Guide (NSF 2014), which specifies that the taxonomic guides are the appropriate standards for wildlife research.

On its face, the PHS FAQ urging the application of a single standard to all [covered] animal care and use seems reasonable and appropriate. However, there exists no single standard that is biologically suitable to all research involving all live vertebrates (and in fact, the policies themselves differ, with the AWA covering only warm-blooded animals). The reality is that the *Guide* says little about wildlife research in the field or in captivity. The section in the *Guide* about field investigations is less than a page in length and overlooks the fact that wild vertebrates are often studied in captivity. Although the *Guide* purports to be applicable to all live vertebrates, domesticated and wild, if it is applied without extensive additional considerations to wild animals, it might not result in the biologically appropriate—and therefore most humane—care. Some specific concerns about applicability of the *Guide* to wildlife research include cage cleaning regimens, frequency of required observations of animals (especially before they have adapted to captivity), social housing, and limitations on euthanasia to methods consistent with the 2013 AVMA *Guidelines for the Euthanasia of Animals*. Also of concern is a "must" (i.e., mandatory) requirement in the *Guide* that "[v]eterinarians providing clinical and/or Program oversight and support must have the experience, training, and expertise necessary to appropriately evaluate the health and wellbeing of the species used in the context of the animal use at the institution" (NRC 2011, 15). The authors estimate that there are no more than 425 wildlife veterinarians in the United States, so the pool of expertise to even approach the expectations of this mandatory requirement is limited.

There is no compelling reason why one standard should apply to all species in all study situations, especially when the biology

and ethical considerations differ substantially between species and types of study. It is worth noting that AAALAC International currently recognizes three standards for accreditation purposes. In addition to the limitations of the *Guide* for wildlife research noted above, restricting PHS Policy to PHS-funded research has the added advantage of reducing regulatory burden by eliminating the necessity for reports to the institutional official or elsewhere when departures from the *Guide* are approved by the IACUC to meet the needs of wildlife activities. The authors reiterate that compliance with PHS Policy is an absolute requirement for all PHS-funded activities but, for the reasons outlined here, we recommend that Assurances be worded so as to delineate clearly between PHS-funded and non-PHS-funded research involving wildlife species.

Do Federal Animal Welfare Policies Require Protocol Review for Wildlife Studies Conducted in the Field?

AWA

The AWA regulations [9 CFR 2.31(d)] provide that field studies are exempt from IACUC protocol review. The definitions section of the regulation (9 CFR 1.1) defines field studies as those "conducted on free-living wild animals in their natural habitat. However, this term excludes any study that involves an invasive procedure, harms, or materially alters the behavior of an animal under study." Neither the statute nor the regulations define these key terms. APHIS provided a clarifying regulation (65 FR 6312, published February 9, 2000, effective March 10, 2000) that amended the definition of field study to make clear that any one of the three conditions would exclude the study from the field study exemption.

PHS Policy

If the research is funded by the PHS or any of the other funding agencies that voluntarily adhere to PHS Policy or that have similar policies (Supplementary Appendix A), all but purely observational studies or other activities that do not alter or influence the activity of the animals must be reviewed and approved. PHS Policy applies to " [a]ny live, vertebrate animal used or intended for use in research, research training, experimentation, or biological testing or for related purposes" (NIH-OLAW 2015b). Although PHS Policy does not address field studies, the accompanying PHS FAQ A.6 (NIH-OLAW 2015a) states:

> If the activities are PHS-supported and involve vertebrate animals, the IACUC is responsible for oversight in accord with PHS Policy. IACUCs must know where field studies will be located, what procedures will be involved, and be sufficiently familiar with the nature of the habitat to assess the potential impact on the animal subjects. If the activity alters or influences the activities of the animal(s) that are being studied, the activity must be reviewed and approved by the IACUC (e.g., capture and release, banding). If the activity does not alter or influence the activity of the animal(s), IACUC review and approval is not required (observational, photographs, collection of feces).

USGP

The USGP (IRAC 1985) was developed by an Interagency Research Animal Committee and is not regulatory in nature. However, the USGP was incorporated into PHS Policy in 1986 and applies "[w]henever US Government agencies develop requirements for testing, research, or training procedures involving the use of vertebrate animals" and "whenever these agencies actually perform or sponsor such procedures." Insofar as the USGP makes no distinction between field studies and research conducted in a laboratory, it should be assumed that the USGP applies to field studies.

Discussion

Both the AWA regulations and PHS Policy are ambiguous. The AWA regulations lack definitions of the key terms "invasive procedure," "harm," and "materially alters behavior," so the interpretations by IACUCs of these terms regarding what constitutes a field study vary widely. Some IACUCs interpret the term "invasive procedure" to mean entry into a body cavity or organ and thus do not include peripheral blood sampling or blood sampling from the quick of a toenail, cloacal and tracheal swabs, or the insertion of a subcutaneous transponder chip.

The term "harm" is extremely broad and almost incapable of application in the absence of a definition. Many IACUCs make the reasonable interpretation that harm means inducing pain or distress—an approach that uses terms that, while not explicitly defined, are consistent with numerous examples given by the Office of Animal Care and Use of the National Institutes of Health in its Guidelines for Preparing USDA Annual Reports and Assigning USDA Pain & Distress Categories. The APHIS Animal Care Policy Manual (USDA 2015a, 11.1) provides guidance as to the meaning of those terms: "A painful procedure is defined as any procedure that would reasonably be expected to cause more than slight or momentary pain or distress in a human being to which that procedure is applied, that is, pain in excess of that caused by injections or other minor procedures." This is the regulatory definition (9 CFR 1.1). However, the regulations do not define the term "distress." The examples given in that policy—food or water restriction or deprivation, noxious electrical shock or thermal stress, paralysis or immobility, forced exercise, and infectious or inflammatory disease models—are largely irrelevant in the context of wildlife research. In the absence of regulatory definitions, IACUCs are left to determine what constitutes harm in the context of wildlife research.

The term "materially alters behavior" is equally vague as to extent and duration and the nature of the outcome by which this condition might be assessed.

Although PHS Policy does not mention field studies, the accompanying FAQ states that the IACUC must know where field studies will be located, know what procedures will be involved, be sufficiently familiar with the nature of the habitat to assess the potential impact on the animal subjects, and review the protocol if the activity alters or influences the activities of the animal(s) that are being studied. However, these key terms are not defined. The examples given in the FAQs that accompany PHS Policy may lead IACUCs to conclude that observations, photographs, and collection of feces will not alter or influence behavior. Wild animals will virtually always be affected by the presence of unknown animals, including conspecifics, competitors, predators, and humans. Generally, the change in behavior is short-lived and has no consequence. The animals will spend more time being vigilant; during this time they will stop feeding, grooming, or undertaking other regular activities. They may hide. They might signal the presence of the intruder, which might alert predators to their location. Without definitions or descriptions, then, the terms used by PHS Policy could actually include virtually all activities involved in wildlife research. Certain activities would seem to be clearly exempt: the use of camera traps, collection of feces, fur samples collected from rubbing sites, and retrieval of molted feathers. Even in these cases, however, animal behavior can be influenced. For instance, rubbing sites are often scent-baited, and the presence of researchers in a bird breeding colony can cause

adults to leave nests or cause nestlings to fledge prematurely. The key in any case would be the nature and duration of the change in behavior or the consequence of the change in behavior.

As to field studies, it is common practice to require the investigator to submit an abbreviated study proposal simply to allow the IACUC to determine if the work does, in fact, constitute a field study. If the IACUC determines that it does not meet the regulatory definition of a field study, the investigator is told that a formal protocol is required.

Note that under both the AWA regulations and PHS Policy, wildlife research conducted on captive animals is subject to IACUC review. (The PHS funding agencies are the following: Agency for Health Care Research and Quality, Agency for Toxic Substances and Disease Registry, Centers for Disease Control and Prevention, Food and Drug Administration, Health Resources and Services Administration, Indian Health Service, National Institutes of Health, and Substance Abuse and Mental Health Services Administration.)

What Species Are Covered?

AWA

" [A]ny live or dead dog, cat, nonhuman primate, guinea pig, hamster, rabbit, or any other warmblooded animal, which is being used, or is intended for use for research, teaching, testing, experimentation, or exhibition purposes, or as a pet. This term excludes birds, rats of the genus *Rattus,* and mice of the genus *Mus,* bred for use in research; horses not used for research purposes; and other farm animals, such as, but not limited to, livestock or poultry used or intended for use as food or fiber, or livestock or poultry used or intended for use for improving animal nutrition, breeding, management, or production efficiency, or for improving the quality of food or fiber. With respect to a dog, the term means all dogs, including those used for hunting, security, or breeding purposes" (9 CFR 1.1).

PHS Policy

"Any live, vertebrate animal used or intended for use in research, research training, experimentation, or biological testing or for related purposes" (NIH-OLAW 2015b).

USGP

The USGP expressly applies to all vertebrate animals (whenever US government agencies develop requirements for testing, research, or research training procedures involving the use of vertebrate animals) (IRAC 1985).

Discussion

If the work is funded by the PHS or any agency that has a regulation or policy that includes all live vertebrates, then all live vertebrates are covered (Supplementary Appendix A). If the agency regulation or policy does not expressly include all live vertebrates, or refers to the AWA regulations, then all warm-blooded animals are covered except rats of the genus *Rattus*, mice of the genus *Mus,* and all birds if the specific animals used were bred for use in research. Farm animals used for research purposes are not covered if the studies are conducted for agricultural or animal husbandry purposes.

Note that with regard to rats, mice, and birds other than those purpose-bred for research, the Animal Care program of the USDA APHIS has prepared a draft regulation, but as of October 2015, the regulation has not been published for comment. In December 2011, APHIS Animal Care announced through a notice to stakeholders that the agency was committed to moving the rule to completion but had to determine how it would implement the regulation upon finalization, particularly those aspects pertaining to birds, and was trying to develop enforcement guidelines that would be feasible and in alignment with existing federal resources.

Do We Need to Inspect Field Sites? If So, How?

A key regulatory requirement that presents difficulty with regard to wildlife research pertains to site inspection.

AWA

Regulations require that the IACUC " [i]nspect, at least once every 6 months, all of the research facility's animal facilities, including animal study areas, using title 9, chapter I, subchapter A-Animal Welfare, as a basis for evaluation; *Provided, however,* That animal areas containing free-living wild animals in their natural habitat need not be included in such inspection" [9 CFR 2.31(c)(1)].

Unless wildlife is brought into captivity for more than 12 hours, the places where individuals are held would not be considered study areas that must be inspected: "*Study area* means any building room, area, enclosure, or other containment outside of a core facility or centrally designated or managed area in which animals are housed for more than 12 hours" [9 CFR 2.31(c)(2)].

PHS Policy

IACUCs are responsible for inspecting " at least once every 6 months all of the institution's animal facilities (including satellite facilities) using the *Guide* as a basis for evaluation." (PHS Policy IV.B.2; NIH-OLAW 2015b). An animal facility is defined as "[a]ny and all buildings, rooms, areas, enclosures, or vehicles, including satellite facilities, used for animal confinement, transport, maintenance, breeding, or experiments inclusive of surgical manipulation. A satellite facility is any containment outside of a core facility or centrally designated or managed area in which animals are housed for more than 24 hours" (PHS Policy III.B; NIH-OLAW 2015b).

Institutions are responsible for oversight of all animal-related activities regardless of how long or where the activity occurs. Satellite facilities (defined by PHS Policy as a containment outside a core or centrally managed area in which animals are housed for more than 24 hours) and areas where any form of surgical manipulations (minor, major, survival, nonsurvival) are performed must be inspected at least once every 6 months by the IACUC as part of the semiannual evaluation (OLAW FAQ E.1; NIH-OLAW 2015a).

OLAW FAQ E.4 (NIH-OLAW 2015a) states:

> While semiannual IACUC inspections of field study sites are not required and in many circumstances are impractical, IACUCs should be apprised of the circumstances under which studies are conducted so that they can consider risks to personnel and impact on study subjects. This may be partially accomplished by written descriptions, photographs, or videos that document specified aspects of the study site. The IACUC should also ensure that appropriate permits are in place. USDA animal welfare regulations exempt areas containing free-living wild animals in their natural habitat from inspection [See 9 CFR, Part 2, Section 2.31(c)(2)].

USGP

The USGP is silent as to inspection of study areas.

Discussion

Under the AWA regulations, field sites need not be visited unless animals are held for more than 12 hours. Even where animals are held for more than 12 hours at a field site, an inspection might serve no useful purpose. Many field sites are temporary; the

field site might simply be a source location where animals are being collected before transport back to an animal care facility or where an animal is being held for 12 hours or more as a planned part of the research or as a consequence of unforeseen circumstances, such as the need to allow recovery from a minor injury. In any case, the animal might very well be released shortly after the 12-hour holding period or well before any inspection is scheduled. It may not be useful to inspect study areas where no animals are being held. IACUC members or the institutional official are likely to see nothing more than a natural setting and find nothing to inspect. If the holding area is sufficiently close to the institution, it may be advisable to schedule an inspection at the time the protocol is approved, asking the researcher to notify the IACUC when animals will be held at the study area.

Under PHS Policy, biannual inspections are required if animals are housed for more than 24 hours or if any surgical manipulations are performed. Because animals are sometimes held for more than 24 hours and because surgeries are conducted in the field, this policy would require biannual inspections of such sites. However, compliance with this requirement is challenging, and, indeed, the OLAW FAQ section E.4 (NIH-OLAW 2015a) states that it is not required and may not be practical. The FAQ offers alternatives: it may be sufficient to include the holding protocols (description of holding device, including size, structure, materials, location, provisions for food and water, protection from predators, monitoring, provision for aseptic measures and the preparation of a sterile field for surgery, and, where appropriate, researcher safety) and to request photographs or video of the device and, where possible, of the animal in the holding device. In actual field settings, it might be prohibitively cumbersome to take photographs or video during procedures. Photo documentation also requires additional personnel. Of greater concern is the fact that taking photos or video may extend the duration of the procedure or distract personnel whose focus should be on the animal. Further, taking a video of procedures goes beyond what site inspection is intended to accomplish. Site inspection is intended to cover the site itself, not the research activities. A video of research activities similarly exceeds AWA requirements. Physical inspection of the field site itself serves no purpose in that field sites are usually ephemeral and there is rarely a structure of any kind to be inspected. Even if a structure is present, an inspection may not be informative unless animals are actually present during the inspection. Further, the limitations imposed by field conditions make it virtually impossible to erect and maintain a structure that would meet the standards required of a laboratory research site or animal holding facility used to house lab animals. For instance, a tent will not have climate and humidity control, and there will be no running water. Further, with the exception of standards for the maintenance of aseptic conditions in surgery, there are no standards by which to assess field sites.

What Standards Are Appropriate to Assess Housing and Husbandry for Wildlife Maintained in Captivity?

AWA

The AWA regulations comprise specific standards for the housing (including sanitation and pest control), feeding, watering, exercise, and transportation of dogs, cats, guinea pigs, hamsters, rabbits, nonhuman primates, marine mammals, and other warm-blooded (9 CFR 3.1–3.142). At the present time there are no specific regulatory standards for wildlife, including birds. Part 3 Standards-Subpart F, which covers warm-blooded species other than dogs, cats, rabbits, hamsters, guinea pigs, nonhuman primates and marine mammals, is written very generally and should be reviewed by the IACUC and the PI. As to bird-specific regulations, the USDA Animal Care program in 2004 published an advance notice of public rulemaking, asking for "comments from the public to help determine how we should regulate the care and use of those animals." In late 2011, a status announcement from Animal Care stated that Animal Care has initiated some revisions to the draft document that will extend the time it takes to publish a proposed rule in the *Federal Register*. The program is currently in the process of clarifying how to implement the regulation upon finalization, particularly those aspects pertaining to birds. Animal Care is committed to moving this regulation forward, but only with enforcement guidelines that are feasible and in alignment with existing federal resources. No further developments have occurred that have been shared with the public.

PHS Policy

The IACUC must inspect all animal facilities, including satellite facilities, at least once every 6 months (PHS Policy IV.B.2; NIH-OLAW 2015b). The *Guide* (NRC 2011) is the official reference standard for facilities under PHS Policy.

USGP

Principle VII (IRAC 1985) states:

> The living conditions of animals should be appropriate for their species and contribute to their health and comfort. Normally, the housing, feeding, and care of all animals used for biomedical purposes must be directed by a veterinarian or other scientist trained and experienced in the proper care, handling, and use of the species being maintained or studied. In any case, veterinary care shall be provided as indicated.

The *Guide* is quite detailed as to environment, housing, and management but is focused on domesticated species.

Discussion

Housing and management standards generally apply only to wildlife when studied in captivity. In the wild, animals are sometimes held for a brief time for processing under the experimental protocol or on the rare occasion where a minor injury occurs during capture and handling that necessitates first-aid measures. Because it can be difficult to obtain and provide suitable food items (with the exception of frugivores, herbivores, or granivores) and because there is stress associated with initial captivity, unless the animal's mobility has been affected, it might be preferable to administer such first-aid as is appropriate, observe the animal for a short while, and then release it. In such cases, some aspects of captive management might foster the animal's well-being if available equipment and conditions permit.

In the context of captive wildlife studies, some aspects of the *Guide* and the AWA regulations (in particular subpart F of part 3, which contains standards for animal health and husbandry, facilities and operating, and transportation for warm-blooded animals other than dogs, cats, rabbits, hamsters, guinea pigs, nonhuman primates, and marine mammals) may warrant consideration. These would include issues such as the maintenance of photoperiod, temperature, and humidity appropriate to the species, as well as ventilation and air quality, noise and vibration, recordkeeping, emergency planning, pest control, and waste disposal. Application, of course, can be challenging. For instance, any wildlife species experiences a wide range of temperature and humidity conditions, particularly across seasons. Further, in an outdoor facility, controlling temperature and humidity can be challenging. Veterinary examination and quarantine are

particular concerns, as is a period of habituation to captivity. It is usually best to refrain from handling and examination while the animal is adjusting to captivity, save for necessary veterinary testing and care and quarantine procedures for eliminating pathogens and parasites. Replicating the social structure for a particular species is challenging in that the introduction of new individuals can be perilous. The animals in the established group may keep a new individual from feeding or may attack or even kill the newcomer. However, individuals of social species might also be stressed when housed alone. Devising ways to introduce newcomers to existing groups without risking the well-being of all may require that social animals be housed alone or with limited contact for a period of time. Conversely, it may be necessary to exceed what would be considered optimal densities to accommodate appropriate social groups. Even basic cleaning and sanitation schedules that would be standard procedure in the context of biomedical research might not be appropriate for wildlife. Animals accustomed to refuges will often create burrows in substrate. Removing substrate removes the burrows and creates stress for the animals until they can create new burrows. Olfactory cues are a fundamental component of the natural environment of most mammals, and husbandry practices should incorporate the maintenance of familiar scents to maximize animal comfort. Individuals frequently scent-mark to establish possession and boundaries of a territory. Regular changing of bedding and washing of the cage and associated equipment eliminates normal scent cues and places captive individuals in a novel and potentially stressful environment. Investigators can reduce stress that accompanies cleaning by changing bedding and cage equipment on a less-frequent cycle than typically used for captive-bred animals. It may be best to leave a portion of the soiled bedding so as to maintain the olfactory cues. Moreover, wild animals will be more sensitive to the disturbance associated with cage-cleaning and human interactions than domesticated species.

The IACUC should rely on the physical status and behavior of the animals to determine if the housing and care are appropriate. Absence of injuries, illnesses, deaths, sustained weight loss, and stereotypical behaviors may be the only way to determine if the care is suitable for the species and for the individuals. The IACUC members assessing facilities for wildlife should consult with wildlife biologists who have successfully kept the same or similar species in captivity or who have the expertise to evaluate the housing and care provided to the animals.

Zoos and aquariums have extensive experience keeping wild animals in captivity, both on exhibit and off exhibit for breeding and research purposes. The American Zoo and Aquarium Association's members have developed Animal Care Manuals that address diet, housing, temperature, humidity, lighting, social environment, and enrichment for a number of taxa. For species not covered by these manuals, consultation with Association of Zoos and Aquariums (AZA) members and the members of the American Association of Zookeepers will likely yield valuable information. The AZA publishes a journal entitled *Zoo Biology*, which includes papers on husbandry, nutrition, and behavior. A new journal entitled the *Journal of Zoo and Aquarium Research* covers (*inter alia*) evidence-based husbandry for living collections with the aim of applying the best available evidence gained from the scientific method.

The AWA largely overlooks the subject of enrichment. PHS Policy does not address enrichment directly but requires PHS-assured institutions to base their programs of animal care and use on the *Guide* (NRC 2011), which addresses the subject of environmental enrichment. *Guidelines to the Use of Wild Birds in Research* (Fair et al. 2010) suggests that, although the practice may be more important for some taxa than for others (e.g., for the Psittaciformes and the Corvidae), it is still important to provide opportunities for natural behaviors such as exercise, foraging, or social interaction to provide new challenges that engage birds cognitively, relieve "boredom," and offset the development of abnormal repetitive behaviors (Meehan et al. 2003). Appropriate enrichment techniques will require an understanding of the animal's natural behavior. Some birds bathe only when it rains, and others will use any small puddle of water. Providing rain-like bathing opportunities will make cage sanitation more difficult, but the absence of bathing opportunities may preclude proper grooming. The appropriate substrate for mammals is often a key element in maintaining their pelage through opportunities for sand bathing to eliminate excess oils. Enrichment might be as simple as providing opportunities to forage for food by catching food throughout the enclosure.

Is the IACUC Required to Review the Manner of Transport of Wild Animals?

AWA

"*Transporting vehicle* means any truck, car, trailer, airplane, ship, or railroad car used for transporting animals (9 CFR 1.1). *Research facility* means any school (except an elementary or secondary school), institution, organization, or person that . . . transports live animals in commerce." The regulations establish transportation standards for dogs, cats, guinea pigs, hamsters, rabbits, nonhuman primates, marine mammals, and other warm-blooded animals, except for birds (9 CFR Subpart 3).

PHS Policy

PHS Policy contains no specific reference to transportation as a component of IACUC review. PHS Policy incorporates guidance regarding transportation of animals of USGP and *Guide* by reference.

OLAW FAQ A.6 (NIH-OLAW 2015a), which pertains to research conducted in the field, states, "When capture, handling, confinement, transportation, anesthesia, euthanasia, or invasive procedures are involved, the IACUC must ensure that proposed studies are in accord with the *Guide* (page 32)."

US Fish and Wildlife Service regulations implementing the 1981 amendments to the Lacey Act [18 USC 42(c)] detail the manner in which live birds and mammals imported into the United States must be handled while in transit (50 CFR 14.101–14.172g). These regulations are essentially a distillation of the much more detailed international standards established by the International Air Travel Association (IATA) in 1993. Those shipping live animals by air should obtain and follow the most recent edition (IATA 2016).

USGP

Principle I (IRAC 1985) states, "The transportation, care, and use of animals should be in accordance with the Animal Welfare Act (7 U.S.C. 2131 et. seq.) and other applicable Federal laws, guidelines, and policies."

The Guide

With regard to wildlife, the *Guide* (NRC 2011) suggests that veterinary input may be needed for projects involving transportation (p. 32) and states, "For wildlife, transportation may occur between the capture site and field holding facilities (p. 107)." Apart from a discussion of regulatory standards and species conservation laws that restrict international movement of some

wildlife, there is no discussion of transportation that is specific to wildlife.

Discussion

Note that caution should be used when relying on the *Guide* for information about regulatory standards and species conservation laws that restrict international movement of some wildlife. The *Guide* (p. 107) contains several inaccurate or incomplete statements on this subject. For instance, it makes mention only of CITES and fails to include the Wild Bird Conservation Act, the Migratory Bird Treaty Act, the Endangered Species Act, the Marine Mammal Protection Act, and the Lacey Act, each of which also restricts international movement of some wildlife. Other text (p. 107) states that the Centers for Disease Control and Prevention and USDA enforce regulations to prevent the introduction, transmission, or spread of communicable diseases and regulate the importation of any animal or animal product capable of carrying a zoonotic disease. In fact, APHIS regulates importation (by permit) only if there is a potential for introduction of a pathogen that can affect agriculture and that does not already occur in the United States as of October 2015. This includes exotic Newcastle disease and highly pathogenic avian influenza (all birds or bird parts); eight species of fish that are susceptible to spring viremia of carp; and foot-and-mouth disease, rinderpest, classical swine fever, swine vesicular disease, African swine fever, and African horse sickness (ruminants, equids, sheep, swine, elephants, rhinos, hippos, and tapirs). The Centers for Disease Control and Prevention regulates the import of dogs, cats, bats, turtles (if the carapace is less than four inches in length), civets, African rodents, and nonhuman primates.

The AWA regulation that details the obligations of the IACUC in terms of protocol review does not specifically mention transport, but if the protocol entails moving the animals in the manner described by the regulation (truck, car, trailer, airplane, ship, or railroad car), then it should be part of the protocol and therefore reviewed. Standards for regulated species exist in part 3 of the AWA regulation. OLAW FAQ D.17 (NIH-OLAW 2015a) is clear on this point. Consultation with a veterinarian familiar with the particular species, or similar species, may indeed be valuable. If chemical restraint or sedation is to be used during transport, veterinary input is essential, particularly if the researcher does not have extensive experience with the species and the pharmaceuticals used.

How to Assess Projects Involving Wildlife

Weighing Objectives of the Study Against Potential Animal Welfare Concerns

AWA

There is no express requirement in the AWA or its implementing regulations that the IACUC weigh the potential harm (to the study animals) versus the potential benefit of the study.

PHS Policy

There is no express requirement in PHS Policy that the IACUC weigh the potential harm (to the study animals) versus the potential benefit of the study. However, PHS Policy endorses the USGP (IRAC 1985), which states, "Procedures involving animals should be designed and performed with due consideration of their relevance to human or animal health, the advancement of knowledge, or the good of society."

In addition, OLAW FAQ D.12 (NIH-OLAW 2015a) touches on a related issue: Is the IACUC responsible for judging the scientific merit of proposals?

> Although not intended to conduct peer review of research proposals, the IACUC is expected to include consideration of the US Government Principles in its review of protocols. Principle II calls for an evaluation of the relevance of a procedure to human or animal health, the advancement of knowledge, or the good of society. Other PHS Policy review criteria refer to sound research design, rationale for involving animals, and scientifically valuable research. Presumably a study that could not meet these basic criteria is inherently unnecessary and wasteful and, therefore, not justifiable.
>
> The primary focus of [scientific review] is scientific merit and the primary focus of the IACUC is animal welfare. The two [reviews] have differing constitutions, mandates and functions. However, since it is not entirely possible to separate scientific value from animal welfare some overlap is inevitable. [Peer reviewers] may raise concerns about animal welfare and IACUCs may question the scientific rationale or necessity for a procedure.

USGP

Principle II (IRAC 1985) states, "Procedures involving animals should be designed and performed with due consideration of their relevance to human or animal health, the advancement of knowledge, or the good of society."

The Guide

The IACUC should consider (*inter alia*) the "rationale and purpose of the proposed use of animals" (p. 25). Further:

> Certain animal use protocols include procedures or approaches that require special consideration during the IACUC review process due to their potential for unrelieved pain or distress or other animal welfare concerns. The topics below are some of the most common requiring special IACUC consideration. For these and other areas the IACUC is obliged to weigh the objectives of the study against potential animal welfare concerns. By considering opportunities for refinement, the use of appropriate nonanimal alternatives, and the use of fewer animals, both the institution and the principal investigator (PI) can begin to address their shared obligations for humane animal care and use. (p. 27).

Discussion

Only the USGP expressly mentions the concept of the benefit of the study. Regardless of the absence of a clear or strong legal mandate, however, it is reasonable to question the necessity for or value of a proposed study. This same issue is implicit in the directive to the IACUC to assure that studies are not duplicated unnecessarily. It is also implicit in the concept of justifying the number of animals to be used. That question ordinarily arises as a matter of statistical necessity, but the underlying premise is that the investigator should not impact more animals than necessary to answer the scientific question. If the study asks no valid or worthwhile scientific question, then there is no justification for using any animals. However, the USGP is very broad in that it considers "advancement of knowledge" and "the good of society" as appropriate standards.

In wildlife biology, the scope of ethical considerations and the parameters that might be used to evaluate benefit-to-cost ratios are very different from those applied to biomedical research. The knowledge acquired in wildlife studies usually benefits the wild taxa that are studied. Knowledge of the basic biology of wild species contributes to virtually all wildlife management and conservation efforts. Wildlife biology is motivated by the need and desire to understand the lives of animals in natural environments. In recent decades, applied research and monitoring to develop appropriate conservation or management strategies has grown to nearly overshadow basic research. In addition, a growing number of studies of fundamental scientific issues in behavioral ecology and ecophysiology are conducted on wild animals under natural conditions, as

scientists have come to understand the limitations of laboratory and captive work in those areas. Whether the primary motivation of field studies is the advancement of scientific knowledge or the acquisition of information used for management purposes, wildlife research yields results that are directly relevant to the welfare and conservation of the species, communities, and ecosystems studied. There have also been numerous instances of wildlife research benefiting humans, as would be the case, for example, with the study of emerging zoonotic disease and disease ecology, the impact of DDT on the food chain, and control of nonnative invasive species, some of which threaten human health.

The enormous variation in field conditions among species or different populations of the same species due to variation in habitat, population size, the presence, number, or type of predators, and other factors makes it important to repeat studies in different places and on different species, so what constitutes repetition in wildlife research must be interpreted cautiously. Longitudinal studies looking at change over time are also critical to our understanding of the natural world, and therefore a study repeated at the same place and on the same species might be valid and important.

How to Implement the "Alternatives" Principles

The 3Rs principles (Russell and Burch 1959) have become central to protocol review and mandatory training under both AWA regulations and PHS Policy. Also known as the alternatives concept, alternative procedures are those that may replace animals with nonanimal methods, reduce the number of animals used, or refine the methodology to minimize animal pain or distress.

These concepts are incorporated into the statutes, regulations, and policies through the protocol review process and in the training requirements. Implementation involves mandatory training for the researchers, and in some cases, a written statement discussing alternatives must be included in the protocol.

Limiting the use of animals entails either replacement with less-sentient animals or nonanimal models or reduction of the number of animals studied (Russell and Burch 1959). The reduction concept is also implicit in the requirement that the "activities do not unnecessarily duplicate previous experiments." Of course, minimizing animal distress (and pain, although not mentioned in this particular section of the regulations) is the essence of the refinement principle.

AWA

The AWA regulatory provisions describing protocol review state:

> (ii) The principal investigator has considered alternatives to procedures that may cause more than momentary or slight pain or distress to the animals, and has provided a written narrative description of the methods and sources, *e.g.*, the Animal Welfare Information Center, used to determine that alternatives were not available; . . . (iv) Procedures that may cause more than momentary or slight pain or distress to the animals will: (A) Be performed with appropriate sedatives, analgesics or anesthetics, unless withholding such agents is justified for scientific reasons, in writing, by the principal investigator and will continue for only the necessary period of time; (B) Involve, in their planning, consultation with the attending veterinarian or his or her designee; (C) Not include the use of paralytics without anesthesia; (v) Animals that would otherwise experience severe or chronic pain or distress that cannot be relieved will be painlessly euthanized at the end of the procedure or, if appropriate, during the procedure; . . . (ix) Activities that involve surgery include appropriate provision for pre-operative and post-operative care of the animals in accordance with established veterinary medical and nursing practices. All survival surgery will be performed using aseptic procedures, including surgical gloves, masks, sterile instruments, and aseptic techniques. Major operative procedures on non-rodents will be conducted only in facilities intended for that purpose which shall be operated and maintained under aseptic conditions. Non-major operative procedures and all surgery on rodents do not require a dedicated facility, but must be performed using aseptic procedures. Operative procedures conducted at field sites need not be performed in dedicated facilities, but must be performed using aseptic procedures [9 CFR 2.31(d)].

The provisions of the AWA regulations pertaining to personnel qualifications state:

> Training and instruction of personnel must include guidance in at least the following areas . . . Utilization of services (e.g., National Agricultural Library, National Library of Medicine) available to provide information: (i) On appropriate methods of animal care and use; (ii) On alternatives to the use of live animals in research; (iii) That could prevent unintended and unnecessary duplication of research involving animals; and (iv) Regarding the intent and requirements of the Act [9 CFR 2.32(c)(5)].

The term "distress" is not defined in the regulations. A "painful procedure is defined as any procedure that would reasonably be expected to cause more than slight or momentary pain or distress in a human being to which that procedure was applied—that is, pain in excess of that caused by injections or other minor procedures" (USDA 2015a, 11.1).

Health Research Extension Act of 1985

> The Director of NIH shall require each applicant for a grant, contract, or cooperative agreement involving research on animals which is administered by the National Institutes of Health . . . to include in its application . . . (1) . . . assurances satisfactory to the Director, NIH that . . . (B) scientists, animal technicians, and other personnel involved with animal care, treatment, and use . . . have available to them instruction or training in the . . . concept, availability, and use of research or testing methods that limit the use of animals or limit animal distress; and (2) a statement of the reasons for the use of animals in research to be conducted with funds provided under such grant or contract [42 U.S.C. 289(d)].

PHS Policy that implements this statutory provision requires the IACUC to determine that procedures with animals will avoid or minimize discomfort, distress, and pain to the animals. Although PHS Policy does not expressly require a written narrative from the researcher describing the search for alternatives, this is customarily the manner in which the IACUC makes this determination. If alternatives are available but are not satisfactory for the proposed research, the investigator is required to explain why the proposed procedures are necessary as opposed to alternatives. To facilitate the efforts of the IACUC to meet its obligations under PHS Policy that procedures undertaken in PHS-funded research will avoid or minimize discomfort, pain, and distress consistent with sound scientific practices, animal protocol submissions should document that investigators have considered alternatives to the use of animals. The assurance must take the form of a written narrative that describes which sources were used to determine that alternatives were not available.

Regarding USGP (IRAC 1985), OLAW FAQ (NIH-OLAW 2015a) states:

> The federal mandate in US Government Principle IV to avoid or minimize discomfort, distress, and pain in experimental animals consistent with sound scientific practices, is synonymous with a requirement to implement refinements (e.g., less invasive procedures or use of analgesia). Similarly, the mandate in US Government Principle III to use the minimum number of animals

> necessary to obtain valid results is synonymous with a requirement to reduce animal numbers. US Government Principle III further states that mathematical models, computer simulation, and in vitro biological systems should be considered, and is synonymous with a requirement to replace non-animal models wherever possible. Thus, consideration of the three "Rs" should be incorporated into IACUC review, as well as other aspects of the institution's program (e.g., investigator training).

USGP

The USGP (IRAC 1985) incorporates the 3Rs in three of the principles:

> III. The animals selected for a procedure should be of an appropriate species and quality and the minimum number required to obtain valid results. Methods such as mathematical models, computer simulation, and in vitro biological systems should be considered.
> IV. Proper use of animals, including the avoidance or minimization of discomfort, distress, and pain when consistent with sound scientific practices, is imperative. Unless the contrary is established, investigators should consider that procedures that cause pain or distress in human beings may cause pain or distress in other animals.
> V. Procedures with animals that may cause more than momentary or slight pain or distress should be performed with appropriate sedation, analgesia, or anesthesia. Surgical or other painful procedures should not be performed on unanesthetized animals paralyzed by chemical agents.

The USGP also requires appropriate training per Principle VIII (IRAC 1985):

> Investigators and other personnel shall be appropriately qualified and experienced for conducting procedures on living animals. Adequate arrangements shall be made for their in-service training, including the proper and humane care and use of laboratory animals.

Although the USGP requires that exceptions to the USGP be reviewed by an appropriate review group, such as an IACUC, the USGP neither requires IACUC review nor prescribes the required content of a protocol to be reviewed by an IACUC. Therefore, it does not require a discussion of alternatives.

The Guide

The *Guide* (NRC 2011, 25–26) reiterates these requirements (*inter alia*,) in saying that the following topics should be considered in the preparation of the protocol by the researcher and its review by the IACUC:

- rationale and purpose of the proposed use of animals
- availability or appropriateness of the use of less invasive procedures, other species, isolated organ preparation, cell or tissue culture, or computer simulation
- justification of the species and number of animals proposed; whenever possible, the number of animals and experimental group sizes should be statistically justified
- unnecessary duplication of experiments
- appropriate sedation, analgesia, and anesthesia

In this regard, the *Guide* goes beyond the AWA and PHS Policy in that it requires that the protocol include a discussion of alternatives whether or not the protocol proposes to use procedures that are likely to cause more than momentary or slight pain or distress. The *Guide* does not carry the weight of law, but given that PHS Policy requires compliance with the *Guide*, this document has come to have quasi-regulatory status. Therefore, even though PHS Policy limits this requirement to situations that are likely to cause more than momentary or slight pain or distress, by incorporating the *Guide* into PHS Policy, PHS has, in effect, made this a requirement for all protocols covered by the PHS Assurance.

Discussion

Under PHS Policy, there is no requirement for assurance, much less documentation, by the researcher that a search for alternatives has been conducted except in cases that involve more than momentary or slight pain or distress. The *Guide* alludes to invasive procedures rather than "momentary or slight pain or distress," and of course, there are noninvasive procedures that may cause more than momentary or slight pain or distress.

It should also be noted that neither the AWA nor the implementing regulations define the term "distress." The regulations define painful procedures as those that "would reasonably be expected to cause more than slight or momentary pain or distress in a human being to which that procedure was applied, that is, pain in excess of that caused by injections or other minor procedures" (9 CFR 1.1). PHS Policy defines neither pain nor distress. The *Guide* defines pain as a "complex experience that typically results from stimuli that damage or have the potential to damage tissue" (p. 120). Distress is defined as "an aversive state in which an animal fails to cope or adjust to various stressors with which it is presented" (p. 121). It can be difficult to recognize this state in wild animals because their responses to aversive conditions vary greatly among species and individuals and because animals are usually held only briefly and are rarely seen again.

In the context of the regulations and policies, we provide a general discussion of the ways that application of the alternatives concepts to wildlife biology will differ from the biomedical context and the reasons for these differences.

Generally, the application of the alternatives principles in the context of wildlife research will look very different than it does in the context of biomedical research. Replacement has come to mean replacement with less-sentient animals, nonanimal models such as cells, tissue culture, and computer-based models. These models may be available and useful in biomedical research. In wildlife research, however, the animals studied are both objects and subjects of the research rather than only the subjects. Replacement is rarely an option in wildlife research. Animal ecologists have modeled some aspects of behavioral ecology, but at some point, hypotheses developed and tested with models are field-tested to determine the extent to which the model explains behavior of actual animals. When studying endangered species, closely related species are sometimes used. Generally, however, replacement is often not an option in wildlife biology.

Reduction calls for methods for obtaining comparable levels of information from the use of fewer animals in scientific procedures or for obtaining more information from a given number of animals so that fewer animals are needed to complete a given research project. The number of animals used should be the minimum number consistent with the aims of the experiment. Achieving this end requires careful statistical planning. Those who lack adequate training in biostatistics should consult a biostatistician to determine the appropriate number of animals or samples needed for the study. A study with an inadequate sample size that results in failure of the study, or a study that cannot be completed or published has actually increased the number of animals used in research without a gain in knowledge.

Refinement entails the use of methods that alleviate or minimize potential pain and distress and that enhance animal well-being. Refinement should be the guiding paradigm for all wildlife research. Refinement entails choosing methods that will generate the information needed while alleviating or minimizing negative impacts. Refinement might be as simple as a reduction in handling time, alternatives to blood sampling to obtain material for genetic sampling, or the use of remote sensing devices in sensitive situations such as the observation of adults with young.

In wildlife biology, the concept of replacement has little relevance. Wildlife research is intended to better understand the basic biology of the species being studied. It is motivated by the need and desire to understand the lives of animals in natural environments. Applied studies are undertaken to develop appropriate conservation or management strategies. In each case, the study species, other species that share their habitats, and often the individual study animals themselves, benefit from the research, directly or indirectly. It would be impossible and pointless to substitute less-sentient animals or nonanimal models. On rare occasions—most often in the context of the study of endangered species—a closely related species might be studied in lieu of another, but even that practice is usually preliminary to a study on the species of interest.

Many research questions can be addressed with the use of samples that do not require the lethal take of an animal or even the capture and handling of live animals. For instance, molted feathers or fur obtained from scratching posts scented to lure mammals can be used, in some cases, to obtain genetic material or for stable isotope or contaminant analysis. Blood samples, saliva, and other fluids can be used for various studies. Some studies may make use of museum specimens, although it should be understood that this option may be limited. For instance, any particular species may be poorly represented, especially as to a particular location. Specimens may be in poor condition. Even today, the online catalogues of museum collections are far from complete, so it can be difficult to find specimens of interest. Permitting and related restrictions make international transport problematic and costly. Most important, characteristics and species distributions change over time, so new voucher specimens are still needed. An excellent example is the impact of climate change on the distribution of various species or the mean size of individuals. Studies in Australia have shown that some species are becoming smaller as the climate warms. Without new specimens to compare with older specimens in museum collections and then, later, to compare with specimens to be collected in the future, this study could not have been done.

Discussions of replacements in wildlife research often focus on the practice of lethal take of individuals for samples or use as voucher specimens. Wildlife biologists are often asked to explain why genetic samples (from blood, tissue, or feathers), alone or in conjunction with photographs, are not adequate substitutes for the collection of a voucher specimen. There are good, practical reasons why photographs are not adequate. Assuming that a photograph can ever adequately show the colors and diagnostic characters of the animal, it does not permit closer examination, even for fundamental questions such as size of anatomical structures. Further, it is difficult to obtain photographs of acceptable quality. The researcher must have a high-quality camera that shoots in RAW format, be skilled in using it, and have adequate batteries, and the camera must not be effected by extreme temperatures or humidity encountered in the field. The lighting must be right (or else the researcher will have to hold the animal waiting for the right light). The researcher will have to have someone else hold the animal while setting up the shot and prevent the animal from escaping. Photographs, feathers, or other tissue samples can never yield data equivalent to complete specimens. Perhaps most important, we never know what materials might be useful for addressing questions as technology evolves, so the value of museum specimens as a source of information for future studies is diminished without complete biological specimens. Studies of contaminants in wildlife often require the use of baseline information from voucher specimens collected decades earlier. Such was the case with DDT. Without eggshells and other tissues collected over many decades, it would have been impossible to identify the increased levels of DDT as a possible cause of eggshell thinning.

The scientific community considers the collecting of a physical specimen to be the best evidence of species identification and location. This is particularly true of the holotypes upon which the International Commission on Zoological Nomenclature (ICZN) recognizes the name to be given to a species or subspecies. The ICZN stated publicly in 2002, "[P]reserved specimens have never been a mandatory requirement, although they have been (and still are) recommended" (Wakeham-Dawson et al. 2002).

Reduction in the number of animals required in any given investigation might be possible, but the inherent variation among wild individuals must be taken into consideration. This variation will, as a matter of statistical validity, require that a greater number of individuals be studied to assure that the sample is representative of the population. This same variation—both among individuals and among conditions in the wild—justifies repeated studies of the same question. Nonetheless, for each new study, the researcher still needs to justify the numbers of animals to be used based on statistical analysis or other considerations.

Refinement is really the only one of the 3Rs that is always applicable to wildlife research. Choosing the method that will generate the information needed while alleviating or minimizing negative impacts is imperative. Refinement might entail a reduction in handling time or the selection of alternate means to obtain material for genetic sampling. Practical concerns may preclude the use of methods that would reduce impact. In some cases, the physical challenges of field research preclude the use of equipment that must be carried long distances or over difficult terrain or that require AC power or specialized storage. Some methods have limited applications. For instance, a new technology to track birds by way of RFID chips on very small, very light-weight geolocators is not useful unless the animal comes within a few centimeters of a reading device. To retrieve data from a data logger, the animal must be recaptured, which is often impossible unless the species exhibits strong site fidelity or has a limited home range. Even then, recapture can be difficult because many individuals become "trap-shy."

Practical Considerations: Field Conditions, Variation among Species

AWA

The AWA regulations do not specify standards.

PHS Policy

In terms of IACUC review, PHS Policy (NIH-OLAW 2015b) mandates compliance with the *Guide:*

> In making this determination, the IACUC shall confirm that the research project will be conducted in accordance with the Animal Welfare Act insofar as it applies to the research project, and that the research project is consistent with the *Guide* unless acceptable justification for a departure is presented.

Moreover, PHS Policy requires that the institution assure PHS that it will comply with PHS Policy. Specifically:

> The Assurance shall fully describe the institution's program for the care and use of animals in PHS-conducted or supported activities. The PHS requires institutions to use the *Guide for the Care and Use of Laboratory Animals* (*Guide*) as a basis for developing and implementing an institutional program for activities involving animals.

OLAW FAQ A.6

OLAW FAQ A.6 states, "Investigators are encouraged to consult relevant professional societies, available guidelines, wildlife biologists, and veterinarians, as applicable, in the design of the field studies (*Guide* page 32, Supplementary Appendix A)" (NIH-OLAW 2015a).

The Guide

The *Guide* (NRC 2011, 32–33) states:

> In preparing the design of a field study, investigators are encouraged to consult with relevant professional societies and available guidelines (Supplementary Appendix A).
>
> The *Guide* does not purport to be a compendium of all information regarding field biology and methods used in wildlife investigations, but the basic principles of humane care and use apply to animals living under natural conditions. IACUCs engaged in the review of field studies are encouraged to consult with a qualified wildlife biologist.

The *Guide*'s Supplementary Appendix A (Additional Selected References) lists, among others, *Guidelines to the Use of Wild Birds in Research, Guidelines of the American Society of Mammalogists for the Use of Wild Mammals in Research, Guidelines for the Use of Fishes in Research,* and *Guidelines for the Use of Live Amphibians and Reptiles in Field and Laboratory Research*.

NSF

The NSF 2013 Grant Proposal Guide (NSF 2014) has explicitly recognized taxon-specific guidelines. On page 13-1, the text now reads:

> 6. Proposals Involving Vertebrate Animals
> a. Any project proposing use of vertebrate animals for research or education shall comply with the Animal Welfare Act [7 U.S.C. 2131 et seq.] and the regulations promulgated thereunder by the Secretary of Agriculture [9 CFR 1.1-4.11] pertaining to the humane care, handling, and treatment of vertebrate animals held or used for research, teaching or other activities supported by Federal awards. In accordance with these requirements, proposed projects involving use of any vertebrate animal for research or education must be approved by the submitting organization's Institutional Animal Care and Use Committee (IACUC) before an award can be made. For this approval to be accepted by NSF, the organization must have a current Public Health Service (PHS) Approved Assurance.
> In the case of research involving the study of wildlife in the field or in the lab, for the provision in the PHS Assurance for Institutional Commitment (Section II) that requires the organization to establish and maintain a program for activities involving animals in accordance with the *Guide for the Care and Use of Laboratory Animals* (*Guide*), the organization has established and will maintain a program for activities involving animals according to the *Guide*. The organization will follow recommendations specified in the *Guide* for details involving laboratory animals, and taxon-specific guidelines approved by the American Society of Ichthyologists and Herpetologists, the American Society of Mammalogists, and the Ornithological Council, as is appropriate for the taxon to be studied.

USGP

Principle I of the USGP (IRAC 1985) states, "The transportation, care, and use of animals should be in accordance with the Animal Welfare Act (7 U.S.C. 2131 *et. seq.*) and other applicable Federal laws, guidelines, and policies."

AAALAC

With limited exceptions that are detailed on the AAALAC website, AAALAC has recognized *Guidelines of the American Society of Mammalogists for the Use of Wild Mammals in Research* (Sikes et al. 2011) and *Guidelines to the Use of Wild Birds in Research* (Fair et al. 2010).

> Reference Resources, first established by AAALAC in 1975, are intended to serve as references and guidelines for laboratory animal care and use programs. Reference Resources are formally reviewed and adopted by the Council and assist site visitors as they evaluate animal programs (AAALAC 2015).

Discussion

Sikes et al. (2012, 832) explain why the use of the taxon-specific guidelines to review wildlife research is appropriate, both historically and biologically:

> Prior to 1986 the welfare of animal subjects in NIH-funded research was overseen by that agency's Office for Protection from Research Risks and focused mainly on the maintenance of research animals in captivity. In 1986, the PHS Policy went into effect, extending oversight to cover experimental procedures. Because the NSF, which funded the most substantial part of wildlife research, required adherence to the PHS Policy, this change also affected wildlife research. At the time, there were no accepted humane policies pertaining to field research. As a result, IACUCs had no appropriate guidance for reviewing protocols for wildlife research and were making widely varying decisions. Recognizing this problem, the NSF urged the presidents of the appropriate scientific societies in 1986 to develop guidelines for the appropriate handling of their taxa. Funding from the NSF facilitated these efforts, and 1987 and 1988 saw the publication of taxon-based guidelines for mammals, birds, reptiles and amphibians, and fishes (Orlans 1988)." In short, these guidelines were solicited by this major federal grant making agency for the express purpose of correcting the absence of information about wildlife in the ILAR *Guide*.

Moreover, the taxon-specific guidelines, which are entirely consistent with the basic principles and legal requirements of animal research, were written and produced in as rigorous a manner as was the *Guide*. They have been extensively revised and peer reviewed by the various professional societies, knowledgeable veterinarians, and other professionals. As detailed by Sikes et al. (2012, 833):

> [But] because the interest of these societies is usually a better understanding of the taxa themselves, their guidelines make extensive use of references and examples directly relevant to field studies and to the well-being of wild populations, as well as that of individuals maintained in captivity. . . . Current editions of society guidelines comprise approximately 333 printed pages (formatting varies widely among the documents) and collectively make reference to 875 articles, the vast majority of them peer-reviewed papers. Of particular importance is that most of the references are focused primarily or exclusively on wild taxa. (Some articles are referenced by more than one set of guidelines or in more than one chapter.) Furthermore, these guidelines are authored and reviewed by individuals with recognized taxon-specific expertise. A search of the 35 authors or editors listed on each of the current guidelines reveals more than 1400 publications indexed by the Web of Science. Even a cursory scan of their titles reveals that most of the papers are focused primarily on the biology of wild species in their native environments or in captivity.

PHS Policy—in particular, the Assurance provision—requires compliance with the *Guide*. However, nothing in PHS Policy prohibits the use of additional resources. The *Guide* (p. 32) counsels consultation with wildlife experts and professional societies. In fact, to fully effectuate the intent of the animal welfare regulations and the alternatives principle, it is necessary to use methods that are appropriate to the biology and ecology of the animals studied. This, in turn, not only justifies, but also makes essential,

an understanding of the need for a much wider range of techniques in wildlife research in the field.

Such work is conducted in uncontrolled and usually uncontrollable environments that differ dramatically from those typical of biomedical research. Whereas vertebrate animals used in laboratory research represent only a few species with well-understood husbandry requirements, there are more than 60,000 species of vertebrates as potential research subjects in wildlife biology. Rather than using animals as models to understand processes related to human disease, the questions asked in wildlife research range from basic aspects of biology, anatomy, and physiology to applied questions focused on conservation or management of entire populations or species. Techniques useful and appropriate for one taxon, experiment, or field situation may be less useful or completely inappropriate in another time, place, or design. Detailed guidelines on husbandry, handling, or even capture techniques are often impossible to generate for groups larger than a few closely related species. The stipulation of specific protocols designed for a specific taxon or situation could actually inhibit humane care and research by imposing requirements inappropriate for the species under study. For these same reasons, the IACUC should recognize the need to test new methods not only for the value to the study questions but also because new methods may have less of an impact (i.e., be faster, less invasive) and thereby constitute a refinement.

Should the IACUC Consider Potential Impacts on the Population?

Unlike biomedical research, wildlife research conducted in the field has the potential to impact animals not actually used in the study. These nontarget animals include the population from which the study animals are drawn and other species that may be affected by the researcher's presence or study methods.

AWA, PHS Policy, USGP, and the Guide

Nothing in the AWA or the implementing regulations, nothing in the Health Research Extension Act or PHS Policy, nothing in the USGP, and nothing in the *Guide* require an IACUC to consider the potential population-level impact of wildlife studies. All are silent on this subject. The very brief discussion of field investigations in the *Guide* (p. 32) says nothing about potential impacts on the population or on other species.

Discussion

Impacts on the study animals, conspecifics in the area that are not included in the study, and other species that happen to be present in the area can range from behavioral responses, such as increased vigilance (usually very brief and of no lasting consequence) resulting from the mere presence of investigator, to demographic changes, such as the permanent removal of one or more individuals from the wild. Removal of a dominant individual may have little impact on population size but may trigger social changes that can affect both individuals and the social group as a whole. Removal or introduction of predators will likely provoke a range of behavioral responses as well as demographic changes as individuals are killed or leave the area. Biosecurity in fieldwork should be considered to minimize potential spread of pathogens from one field site to another by contaminated clothing or equipment in the course of conducting the research. The introduction of novel pathogens between study sites could impact animal populations and cause harm, pain, or distress to individuals. For instance, white nose syndrome, a fungus that kills bats, may have been introduced to North America and spread by human activities, so recommended practices now include the use of disposable clothing and equipment or disinfection of these items before use at other sites to avoid spreading this pathogen.

Despite the absence of a regulatory or policy requirement, many IACUCs elect to delve into the question or potential impacts on the population. Arguably, it could be construed as a logical corollary of the requirement to use the minimum number of animals necessary to obtain valid results. However, other animals that happen to be present are not actually being used in the study. Perhaps an argument could be made that if the study happens to also include a census of the other animals present at the time the study is conducted, that is, a count of the number of predators during a study of breeding success, then the animals counted during the census are being used.

As a practical matter, however, this inquiry is likely to have little value. Population-level impacts are difficult to predict because the researcher may not have sufficient knowledge of population sizes and species interactions. There may be no published information, and a census, even if possible or practical, will not yield sufficient information. A single census at a given point in time will not produce useful population estimates because wild populations can fluctuate widely over seasons and years. IACUC review of these concerns would also require that the IACUC have sufficient understanding of quantitative population biology to assess the available data. Furthermore, population sizes change for a wide variety of reasons, and it would be virtually impossible to attribute a change in population size to a given research protocol except in the most extreme cases, such as the deliberate removal of the entire population. Even then, the effect may be short-lived as individuals from surrounding areas populate the vacated habitat and make use of available resources. In other words, another population or segment of a population may actually benefit and grow as a result of an activity that depresses the size of a nearby target population.

For research that requires permits, the issuance of federal or state permits and land management permits address these concerns. These permits are issued by agency staffers who have at least knowledge of overall population status and trends. Permit approval means that these individuals have determined that the extent of take needed for the study will not be detrimental to the population or that any population-level impact is justified by the value of the knowledge to be gained. Instances where permits are issued at multiple levels provide even more assurance to the IACUC because receipt of these permits necessarily means that at least two different agencies, one at a federal level and the other usually more local level, have considered the potential impacts.

The researcher has no control over the timing of the issuance of permits; it is possible, if not likely, that the permits will not have been issued at the time of the protocol review. PHS Policy does not allow conditional, contingent, or provisional approval. If the protocol as submitted documents the submission of permit applications and the IACUC finds it to be acceptable, the best practice for an IACUC would be to indicate in writing to the investigator that the protocol has been approved but that the animal work is not to begin until required permits are obtained. The interval between application and issuance can vary greatly. If the researcher has not applied for a permit at least 30 days before the planned start date of the research, it is unlikely that the permit will be issued by that planned start date. However, some permits are, by regulation, automatically continued if a renewal application is filed at least 30 days before the expiration date. Further, it is always possible that the permit will be issued more

expeditiously than is normally the case, that is, within 30 days or very shortly after the planned start date. Therefore, it is reasonable for the IACUC to review the protocol even if it seems unlikely that the permit will be issued before the planned start date, subject to the requirement that the work may not start until all permits are obtained.

Where and How Can Researchers Obtain Adequate Training?

AWA

> It shall be the responsibility of the research facility to ensure that all scientists, research technicians, animal technicians, and other personnel involved in animal care, treatment, and use are qualified to perform their duties. This responsibility shall be fulfilled in part through the provision of training and instruction to those personnel. Subsection (b) details the specific training that is required: Training and instruction shall be made available, and the qualifications of personnel reviewed, with sufficient frequency to fulfill the research facility's responsibilities under this section and § 2.31.(c) Training and instruction of personnel must include guidance in at least the following areas: (1) Humane methods of animal maintenance and experimentation, including: (i) The basic needs of each species of animal; (ii) Proper handling and care for the various species of animals used by the facility; (iii) Proper pre-procedural and post-procedural care of animals; and (iv) Aseptic surgical methods and procedures; (2) The concept, availability, and use of research or testing methods that limit the use of animals or minimize animal distress; (3) Proper use of anesthetics, analgesics, and tranquilizers for any species of animals used by the facility [9 CFR 2.32(a)].

PHS Policy

PHS Policy states, "Personnel conducting procedures on the species being maintained or studied will be appropriately qualified and trained in those procedures" (PHS Policy IV.C.1.f; NIH-OLAW 2015b).

USGP

Principle VIII (IRAC 1985) states: "Investigators and other personnel shall be appropriately qualified and experienced for conducting procedures on living animals. Adequate arrangements shall be made for their in-service training, including the proper and humane care and use of laboratory animals."

The Guide

The *Guide* includes the review of the adequacy of training as a component of the protocol review. It also states that staff should receive training and/or have the experience to complete the tasks for which they are responsible (p. 16).

Discussion

There is little formal training specific to the techniques of wildlife research available. Training as to specific methods is nearly always a matter of demonstration by an experienced researcher followed by hands-on but supervised learning. It is often conducted on the job. When the experienced researcher determines that the individual has adequate skills, supervision ceases.

IACUCs should encourage researchers to share details regarding novel techniques and procedures with colleagues. However, some formal training opportunities in the more commonly used techniques are available. These include a series of manuals and a training and certification program through the North American Banding Council, although the actual training is conducted on an individual basis, as described above. There are a handful of organized training courses, such as the field ornithology course taught at the Shoals Marine Laboratory (operated by Cornell University) or the courses offered by the Organization for Tropical Studies. There are also some specialized training programs such as those offered by the Institute for Bird Populations. Colleges and universities with programs in wildlife management often offer courses in field techniques. However, IACUCs should recognize that it is far more common for field biologists to receive one-on-one training from more experienced individuals, either as students or, after becoming an independent researcher, from colleagues, because of the enormous range and variation in types of procedures, including species-specific modifications. Rarely are these individuals veterinarians.

Some of the training requirements listed in the AWA regulations may be unnecessary for many wildlife researchers. Many wildlife biologists will never conduct surgery or attempt procedures for which the use of analgesics, anesthetics, or tranquilizers would be necessary. Generally, tranquilizers are used in the context of capture only when no other means of capture and handling that is safe for the animal and the researchers are available, which is usually the case only for carnivores or larger species, especially ungulates. If, however, such techniques are to be used, appropriate training is required, as would be true of any technique. Except for care administered immediately after a procedure while the animal is still sedated, there is rarely postprocedural care for wild animals studied in the field because, in most cases, the animals are not seen after they revive and are released. They are rarely recaptured.

With regard to training in the regulations specific to wildlife research, there is extensive information available in *Guidelines to the Use of Wild Birds in Research* (Fair et al. 2010) that is pertinent for other taxa as well. A Collaborative Institutional Training Initiative course designed specifically for work with wildlife was released in 2013 (CITI 2015).

What Role Does the IACUC Have in Investigator Safety/Health Issues in Wildlife Biology?

AWA

The regulations are silent as to the issue of researcher safety and health.

PHS Policy

The assurance provided by the institution to PHS must describe "the health program for personnel who work in laboratory animal facilities or have frequent contact with animals" (PHS Policy IV.A.1.f; NIH-OLAW 2015b).

USGP

The USGP does not contain provisions pertaining to researcher safety and health.

The Guide

The *Guide* states that each institution must establish and maintain an occupational health and safety program as an essential part of the overall program of animal care and use (p.17). In fact, the legal reference in question is the Occupational Safety and Health regulation promulgated by the US Department of Labor, and the specific sections cited in the *Guide* are 29 CFR 1910, subparts A (radiation), G (occupational health and environmental control), and I (personal protective equipment).

Discussion

A reading of the authorities cited above leaves no doubt that the law does not require that the IACUC address occupational safety and health issues. The question of researcher safety and health may be more appropriately handled by the institution's risk management staff than by the IACUC or through interaction between

the IACUC and occupational health and safety units. The hazards of field research are diverse, and it is unlikely that most IACUC members have the expertise to identify those hazards, much less evaluate the adequacy of the investigator's health and safety planning. However, many smaller institutions do not have risk management experts on staff. To a large extent, the risk management staff or the IACUC members must rely on the researcher's expertise in identifying the potential risks of a research project. These risks include zoonotic disease, encounters with dangerous or venomous wildlife, risks associated with the means of transport, and even weather conditions.

In reality, many institutions delegate this responsibility to the IACUC. In some cases, it is an administrative function in that the IACUC need only determine that the researcher has interacted with the institution's risk management or occupational health and safety offices, informed that authority of potential risks, and received approval of the health and safety plan that addresses that risk. In other cases, institutions actually rely on IACUC members to address the substance aspects of the health and safety issues. This approach can be problematic in that it puts the IACUC members in the position of using their authority under the AWA or responsibility under PHS Policy to refuse protocol approval for a reason unrelated to the AWA or PHS Policy.

Most of the specific regulations discussed in the *Guide* have limited relevance to wildlife biology except for the identification of potential hazards, measures to avoid those hazards (including personal protective equipment), first aid, and emergency planning, including communication, transportation, and insurance coverage.

With regard to zoonotic disease, the measures that should be taken to avoid contracting a zoonotic disease and to avoid transmitting it to others should be commensurate with the extent of the risk and of the consequence of contracting the disease. The Ornithological Council has published a peer-reviewed fact sheet (Ornithological Council 2010) that differentiates among various zoonotic pathogens transmitted by birds and the measures appropriate to the risk posed by each of them. The American Society of Mammalogists has guidelines (Kelt et al. 2010) pertaining to hanta virus. Preventive measures, including personal protective equipment, can be cumbersome and interfere with research techniques, especially under field conditions. However, if there is a reasonable likelihood of encountering a pathogen that has the potential to cause serious disease, more extensive measures are warranted, even if burdensome, uncomfortable, or costly. Diseases of concern might be associated with the study animals or present in the ambient environment. These include arthropod-borne pathogens (such as Lyme or erlichiosis), pathogens present in water (such as giardia), avian pathogens (such as chlamydiosis), and mammalian pathogens (such as Hanta).

Protocols should include a discussion of vaccinations or other prophylaxes and preventative measures and a plan to recognize symptoms and determine when the researcher or field technicians should seek medical assistance.

Postapproval Monitoring

AWA

"The IACUC shall conduct continuing reviews of activities covered by this subchapter at appropriate intervals as determined by the IACUC, but not less than annually" [9CFR 2.31(d)(5)].

PHS Policy

"The IACUC shall conduct continuing review of each previously approved, ongoing activity covered by this Policy at appropriate intervals as determined by the IACUC, including a complete review in accordance with IV.C.1-4 at least once every three years" (PHS Policy IV.C.5). In addition, the mandatory reporting requirements (PHS Policy IV.B.3–) necessarily entail monitoring of the research activity after the protocols have been approved (NIH-OLAW 2015b).

USGP

The USGP contains no provisions pertinent to postapproval monitoring.

The Guide

"Continuing IACUC oversight of animal activities is required by federal laws, regulations, and policies. A variety of mechanisms can be used to facilitate ongoing protocol assessment and regulatory compliance. Postapproval monitoring (PAM) is considered here in the broadest sense, consisting of all types of protocol monitoring after the IACUC's initial protocol approval" (NRC 2011, 33).

Discussion

The two-page discussion of postapproval monitoring (PAM) in the *Guide* (pp. 33–34) is very general; it notes that federal laws require continuing oversight. The *Guide* itself does not mandate PAM or any particular mechanism nor does it require inspection or observation.

Inspection or observation by IACUC members of wildlife research activities is generally not feasible for wildlife studies conducted in the field unless the research involves captive animals or unless there is a stationary field site in relatively close proximity to the institution. It is usually difficult and time-consuming to visit even field sites that are relatively close to the institution. Inclement weather may intervene, field technicians may be scattered across numerous sites, or the animals that are to be studied may simply not be found on the day that observation is to take place. The cost of PAM is rarely covered by the direct or indirect costs portion of a grant. This is a serious barrier to observation of research activities at distant sites. The larger problem is that veterinary staff and IACUC members often are not trained in field methods and have difficulty assessing the research activity.

Some IACUCs ask the researcher to carry a video camera into the field to record a portion of the field activities. As equipment has become smaller and lighter—even a smart phone may suffice for this purpose—this idea is less burdensome than it was only a few years ago. However, recording may require that an extra person be present so that recording will not interfere with animal handling and manipulation. In addition, it may be preferable to reserve battery use for emergencies.

For studies conducted in the field, then, it may be necessary to assess compliance with the approved protocol and appropriate handling and care by way of a reporting form that calls for the investigator to list and detail each instance of deviation from the protocol and the reason for said deviation. The *Guide* specifically lists "regular review of adverse or unexpected [experimental] outcomes affecting the animals" as an effective monitoring strategy.

Should Wildlife Studies Be Included on APHIS Annual Reports (Form 7023)? What Are the Reporting Requirements Regarding Wildlife Research under PHS Policy?

AWA

The regulation (9 CFR 3.26) requires the submission of an annual report by each "segment of the research facility, or that department, agency, or instrumentality of the United States, that uses

or intends to use live animals in research, tests, experiments, or for teaching." The report must include (*inter alia*) a list of the numbers of animals upon which teaching, research, experiments, or tests were conducted involving:

- no pain, distress, or use of pain-relieving drugs;
- accompanying pain or distress to the animals and for which appropriate anesthetic, analgesic, or tranquilizing drugs were used;
- accompanying pain or distress to the animals and for which the use of appropriate anesthetic, analgesic, or tranquilizing drugs would have adversely affected the procedures, results, or interpretation of the teaching, research, experiments, surgery, or tests; and
- an explanation of the procedures producing pain or distress in these animals and the reasons such drugs were not used shall be attached to the annual report.

This report is submitted on APHIS Form 7023.

Discussion

In February 2015, APHIS Animal Care issued a revision of its Animal Welfare Inspection Guide (USDA 2015b). The revision makes clear (at 7–47) that the Annual Report should not report any animals used for the following:

> Field studies which meet the following criteria and are therefore exempt from the regulations and do not require a written, approved exemption. The study does not: [1.1, 2.31(d)(1)]
>
> Harm the animals under study
>
> Involve an invasive procedure
>
> Materially alter the behavior of the animals under study
>
> Animals euthanized, killed, or trapped, and collected, such as for study or museum samples, from their natural habitat via humane euthanasia.

(See discussion in the next section and note that the terms used by Animal Care in the Inspection Guide, quoted here, are not aligned with the AVMA Euthanasia Guidelines or the taxon societies' suggested application of those terms in the context of wildlife research.)

The terms in the definition of field study are likewise undefined, leading to considerable uncertainty among IACUCs and varying interpretations. However, it is clear that if an IACUC classifies a particular protocol as a field study, then animals studied under that protocol are not to be included on the annual report.

The question then becomes which category is appropriate for animal capture and noninvasive field procedures. The Ornithological Council and the American Society of Mammalogists requested in June 2010 that the APHIS Animal Care program issue guidance addressing these questions. In making that request, these two societies submitted a position statement regarding the appropriate classification of animal capture and noninvasive field procedures:

> *USDA classifications as applied to animal capture and non-invasive field procedures*
>
> Mammal capture devices are designed either to hold the animal unharmed (live-traps) or to kill the animal outright upon capture. The Guidelines of the American Society of Mammalogists for the Use of Wild Mammals in Research discuss appropriate methods and trap types for capturing or collecting free-ranging mammals (Sikes et al. 2011).
>
> Birds can be captured with a variety of devices, all designed to capture and hold a bird unharmed until released. Although scientific collecting of birds may sometimes entail capture of a live bird followed by euthanasia, the capture methods themselves are not intended to be lethal and in fact do not kill birds. The 2010 revision of the *Guidelines to the Use of Wild Birds in Research* discusses capture methods and the practices needed to assure that capture does not result in harm to birds (Fair et al. 2010).
>
> Barring mechanical malfunctions and with appropriate placement and trap checking frequency, animals captured in live-traps or nets are simply held without injury until removal. Appropriate training is essential for setting capture devices and for removing animals from those devices. Pain or distress, as described in the APHIS Animal Care Resource Guide, is unlikely to result from the simple capture of free-ranging mammals or birds using most live traps or capture techniques covered in the American Society Mammalogists or Ornithological Council Guidelines, so animal usage in these instances is consistent with USDA category C.
>
> Most tissue sampling and marking techniques in the field also are consistent with USDA pain category C provided that procedures are not more invasive than peripheral blood sampling. Support for this classification is provided in the *Guidelines for Preparing USDA Annual Reports and Assigning USDA Pain and Distress Categories*. This document is distributed by the NIH Office of Animal Care and Use, which is the oversight office for intramural research. This guidance expressly states that Category C includes most blood and tissue collection procedures that involve no or only momentary or slight pain. Based on these same NIH Guidelines, USDA category C is also appropriate in instances where protocols requiring peripheral tissue sampling or tagging and release of free-ranging animals necessitate chemical immobilization to conduct the procedures provided that immobilization is performed only to facilitate the procedure and protect the animal and the researcher from injury rather than to alleviate pain or distress induced by the procedure.
>
> Free-ranging mammals captured in live traps and subsequently euthanized as part of the research study or that are taken in properly functioning kill-traps meet the standards for either USDA category C or D; the distinction between these reporting categories depends upon how the animal dies. Animals taken in live traps that show no obvious signs of pain or distress and subsequently euthanized using accepted methods that avoid inducing pain or distress and those taken in properly functioning kill traps fit the definition for reporting under USDA category C. This conclusion is consistent with example #4 in the USDA APHIS Research Facility Inspection Guide (section 14.1.10) except that death is intentional rather than unexpected. The Research Facility Inspection Guide pertains to laboratory animals rather than free-ranging wildlife, but euthanasia following a live capture that does not result in pain or distress is analogous to this example.
>
> The *Guidelines for Preparing USDA Annual Reports and Assigning USDA Pain and Distress Categories* make clear that assignment of animals to a reporting category is done on a retrospective basis. Even though a trapping method might ordinarily comprise Category C, if a problem occurred in the field that resulted in pain or distress necessitating pain alleviation, then Category D is the appropriate reporting category for that particular animal. If live-trapping brings about pain or suffering that necessitates euthanasia, or if kill-trapping fails to bring about swift death and leaves a conscious animal in pain or distress, category D is again the appropriate reporting category. These situations are analogous to example #3* APHIS Research Facility Inspection Guide depending upon trap type, trap specificity, and trapping technique.

Note that as of 2015 this is example #4 in the APHIS Research Facility Inspection Guide (USDA 2015b).

PHS Policy

Generally, "The IACUC shall conduct continuing review of each previously approved, ongoing activity covered by this Policy at appropriate intervals as determined by the IACUC, including a complete review in accordance with IV.C.1-4. at least once every three years" (PHS Policy IV.C.5; NIH-OLAW 2015a).

Specific reporting requirements (PHS Policy IV.F.3; NIH-OLAW 2015a) are:

(a) any serious or continuing noncompliance with this Policy;
(b) any serious deviation from the provisions of the *Guide*; or
(c) any suspension of an activity by the IACUC.

PHS Policy applies to "[a]ny live, vertebrate animal used or intended for use in research, research training, experimentation, or biological testing or for related purposes." PHS Policy is silent as to field studies; it neither excludes nor includes field studies. However, the OLAW FAQ A.6 (NIH-OLAW 2015a) states:

> If the activities are PHS-supported and involve vertebrate animals, the IACUC is responsible for oversight in accord with PHS Policy. IACUCs must know where field studies will be located, what procedures will be involved, and be sufficiently familiar with the nature of the habitat to assess the potential impact on the animal subjects. If the activity alters or influences the activities of the animal(s) that are being studied, the activity must be reviewed and approved by the IACUC (e.g., capture and release, banding). If the activity does not alter or influence the activity of the animal(s), IACUC review and approval is not required (observational, photographs, collection of feces).

Discussion

Activities that do not alter or influence the activity of the animals are exempt from IACUC review and approval of the protocol. Even in the absence of a protocol, however, a deviation from the provisions of the *Guide* could occur, except for the fact that there are essentially no provisions in the *Guide* that pertain to wildlife research and those that are present cover only general principles. Investigators and IACUC members alike should be sensitive to any changes to the planned study methods or unanticipated impacts on the study animals that would eliminate a project from the field studies exemption. The IACUC is the authority as to whether the study qualifies for exemption as a field study, so the investigator should notify the IACUC of the changes and give them the opportunity to reassess the exemption decision. All wildlife research that is subject to IACUC review and approval is subject to the reporting requirements except as noted in the USDA Facilities Inspection Guide (USDA 2015b).

Special Consideration: Euthanasia in Wildlife Research

AWA

"(xi) Methods of euthanasia used must be in accordance with the definition of the term set forth in 9 CFR part 1, § 1.1 of this subchapter, unless a deviation is justified for scientific reasons, in writing, by the investigator" [9 CFR 2.31(d)(xi)]. Section 1.1 states, "*Euthanasia* means the humane destruction of an animal accomplished by a method that produces rapid unconsciousness and subsequent death without evidence of pain or distress, or a method that utilizes anesthesia produced by an agent that causes painless loss of consciousness and subsequent death."

PHS Policy

"g. Methods of euthanasia used will be consistent with the recommendations of the American Veterinary Medical Association (AVMA) Panel on Euthanasia unless a deviation is justified for scientific reasons in writing by the investigator" (PHS Policy IV. C.g; NIH-OLAW 2015b). OLAW FAQ A.6 (NIH-OLAW 2015a) pertaining to research conducted in the field states, "When capture, handling, confinement, transportation, anesthesia, euthanasia, or invasive procedures are involved, the IACUC must ensure that proposed studies are in accord with the *Guide*."

USGP

The USGP contains no provisions specific to methods of euthanasia.

The Guide

The Guide states, "Unless a deviation is justified for scientific or medical reasons, methods *should* [emphasis added] be consistent with the AVMA Guidelines on Euthanasia" (p. 132, 2007 or later editions).

AVMA Guidelines

The AVMA revised its euthanasia guidelines in 2013 (AVMA 2013). With regard to free-ranging wild animals, this edition

> updates and expands on previous editions by recognizing an inherent lack of control over free-ranging wildlife, accepting that firearms may be the most appropriate approach to their euthanasia, and acknowledging that the quickest and most humane means of terminating the life of free-ranging wildlife in a given situation may not always meet the criteria established for euthanasia (i.e, distinguishes between euthanasia and methods that are more accurately characterized as humane killing (AVMA 2013, 81)).

Discussion

The 2013 AVMA Guidelines recognize that humanely terminating the lives of free-ranging wild animals presents challenges due to lack of direct control of the animals and potential danger and distress for both subject animals and humans when using techniques that bring personnel in close proximity to the animals. The AVMA also acknowledges that the diversity of situations that may be encountered makes it "difficult to strictly classify methods for termination of free-ranging wildlife as acceptable, acceptable with condition, or unacceptable" (pg 81). The guidelines go on to state, however, that "these acknowledgments are not intended to condone a lower standard for the termination of wildlife" pg 81). The revision distinguishes between euthanasia as a means of ending suffering and the use of humane techniques to end life, whether to end suffering or for other reasons. Although the guidelines do not say so explicitly, this second category is presumably the same thing as "humane killing." The document states further:

> While recommendations are made, it is important for those utilizing these recommendations to understand that, in some instances, agents and methods of euthanasia identified as appropriate for a particular species may not be available or may become less than an ideal choice due to differences in circumstances. Conversely, when settings are atypical, methods normally not considered appropriate may become the method of choice. Under such conditions, the humaneness (or perceived lack thereof) of the method used to bring about the death of an animal may be distinguished from the intent or outcome associated with an act of killing. *Following this reasoning, it may still be an act of euthanasia to kill an animal in a manner that is not perfectly humane or that would not be considered appropriate in other contexts* [emphasis added]. For example, due to lack of control over free-ranging wildlife and the stress associated with close human contact, use of a firearm may be the most appropriate means of euthanasia. Also, shooting a suffering animal that is *in extremis*, instead of catching and transporting it to a clinic to euthanatize it using a method normally considered to be appropriate (e.g., barbiturates), is consistent with one interpretation of a good death. The former method promotes the animal's overall interests by ending its misery quickly, even though the latter technique may be considered to be more acceptable under normal conditions (Yeates 2010). Neither of these examples, however, absolves the individual from his/her responsibility to ensure that recommended methods and agents of euthanasia are preferentially used (AVMA 2013, 7).

Conditions in the field require careful planning to use methods considered by the AVMA to be acceptable or acceptable with conditions, if feasible at all. If a wildlife veterinarian with relevant experience (i.e., with similar species and similar conditions) is available, the consultation is advisable. Even with great planning, other methods for humane killing may be necessary, which would require review and approval by the IACUC. Only recently have veterinarians been able to legally transport and use Drug Enforcement Administration (DEA)–controlled substances away for their registered location. The Veterinary Mobility Act, signed into law in 2014, allows veterinarians (but not other DEA registrants) to legally transport and use controlled substances for the provision of veterinary care. This act will facilitate some field studies that have the luxury of having a veterinarian participate in the study. However, most studies do not include veterinarians. Even non-DEA-controlled pharmaceuticals are often unavailable except through a veterinarian who may be reluctant to provide them to others because most states require a Veterinary-Client-Patient Relationship, which rarely exists in the case of wildlife research. Universities concerned about liability may prohibit the use of veterinary pharmaceuticals except by a veterinarian. Export outside of the United States may be restricted. A great deal of wildlife research takes place outside the United States, and the pharmaceuticals that are considered acceptable or acceptable with conditions by the AVMA might be illegal, unavailable, or available only to veterinarians who are residents in the country where the research will take place. Inhalants can be problematic because even portable delivery systems (such as vaporizers and face masks) can be cumbersome and impossible to transport to remote locations. In some cases, expeditions extend for weeks and involve foot travel in remote conditions. Some inhalants, such as isoflurane, can be administered on saturated cotton balls placed in closed jars, much as ether was once used. This is a simple system. However the operator must be trained and demonstrate proficiency in their use and how to assess unconsciousness and confirm death. Thoracic compression (or, as to birds, cardiac compression) or another physical method of euthanasia could/should be applied to an animal euthanized by an inhalant method to assure death; this combined method is considered acceptable by the AVMA. The jar should not be opened unless the animal is insentient.

Some wildlife research requires an intact carcass free of introduced substances. Intact carcasses are particularly important for taxonomic studies or any study that requires a voucher specimen. In these cases, physical methods such as decapitation or cervical dislocation—which can result in the inadvertent separation of the head from the body and distort standard body measurements—are unsuitable. Because tissues are often used in genetic biochemical analyses, it is important to avoid introducing chemical substances. Because these studies often take place in remote areas, physical methods may be the only alternative, including gunshot with suitable ammunition and shot placement or thoracic compression (or, as to birds, cardiac compression). The 2013 AVMA Guidelines note that, although it has been extensively used in the field, thoracic compression was classified as an unacceptable means of euthanasia and data supporting its use were lacking concerning the degree of distress it induced and time to unconsciousness or death. The Ornithological Council and the American Society of Mammalogists expressed concern to the AVMA because it seemed likely that IACUCs concerned about departures from the *Guide* would refuse to approve protocols involving thoracic compression (cardiac compression when used on birds), even in circumstances where no other method was suitable. In response, the AVMA issued a Literature Review, which notes (consistent with PHS Policy) that " when scientifically justified, the IACUC has and should employ the authority to approve killing techniques not listed as recognized forms of euthanasia. This might include approving thoracic compression where it represents the most humane option available or practicable, or approving the use of drugs with analgesic properties that may not be scheduled drugs" (AVMA 2011). This document goes on to state that "thoracic compression should not be prohibited where its use is necessary to minimize animal suffering or is scientifically justified (such as under the oversight of an Institutional Animal Care and Use Committee." The Ornithological Council has issued a fact sheet and position statement that are available upon request for those who would like more detailed information about thoracic compression (or, as to birds, cardiac compression) when assessing protocols that propose to use this method. It is important to note that when AAALAC International adopted the AVMA Guidelines for the Euthanasia of Animals, they did so with a single exception—the justified use of thoracic compression in studies of wild birds and mammals (AAALAC 2015).

Wildlife research often provides serendipitous opportunities to capture animals that represent important specimens while investigators are conducting other research. This might entail finding an animal out of its known range or finding animals not thought to be present at a particular field site. In such instances, and provided their permits and protocols allow such, investigators should plan for the unexpected in the best manner possible and submit protocols that include a variety of methods of euthanasia or humane killing suitable to the species they might encounter and appropriate under the circumstances.

For IACUC purposes, the fact that the *Guide* states that "[u]nless a deviation is justified for scientific or medical reasons, methods *should* [emphasis added] be consistent with the AVMA Guidelines on Euthanasia" is significant. According to OLAW, this specifically described *Guide* exception is not a deviation from a "should" statement if justified for scientific reasons and approved by the IACUC and thus is not a departure from the *Guide* (NIH-OLAW 2013) and is not included either on the semiannual report to the Institutional Official or to OLAW.

For wildlife research conducted in captivity, the AVMA Guidelines generally pose no problem because a veterinarian can be present or can make available the pharmaceutical agents that are considered acceptable or conditional in the 2013 edition. For inhalant and noninhalant methods, the equipment and agents are usually available. The purpose of the research may require the use of a physical method, but generally, anesthesia will be available for use to ameliorate pain and reduce or eliminate fear.

Finally, protocols should address carcass disposal if any part of the carcass is to be left in the field. Secondary toxicity caused by injected anesthetics, euthanasia solutions, or lead ammunition used for lethal gunshot can occur if carcasses are improperly disposed of or left for scavenging.

Special Consideration: Release of Wildlife Held in Captivity

AWA

The AWA contains no provision pertaining to the release of wildlife studied in captivity.

PHS Policy

PHS Policy contains no provision pertaining to the release of wildlife studied in captivity.

USGP

The USGP contains no provision pertaining to the release of wildlife studied in captivity.

The Guide

"When species [sic] are removed from the wild, the protocol should include plans for either a return to their habitat or their final disposition, as appropriate" (NRC 2011, 32). (Note that researchers do not remove species from the wild; researchers remove one or more individual animals.)

Discussion

Generally, the release of animals back to the wild after they have been held in captivity for research is not advisable unless study design or conservation concerns warrant their repatriation. However, this is a species-specific consideration. It can be difficult for individuals of some species to regain the physical condition and normal behaviors needed for survival in the wild. In this regard, the duration of the captivity is also a factor. The shorter the duration, the less likely it is that the individuals will lose their physical conditioning or will develop behaviors that are maladaptive to survival in the wild. The type of housing will play a role. Birds that have been unable to fly for some period of time may lose mass in the flight muscles or become obese. Before planned release, researchers should observe the individuals closely to assess changes in physical condition and behavior.

It is likely that other individuals will have replaced those removed from the wild, so the resources at the release site may not be sufficient to support additional animals. The natural habitat at the release site may have been altered or eliminated. Release at another site not known to those individuals may make it difficult for the individuals to find resources, and they may be precluded from obtaining resources by conspecifics. This is particularly true of highly social species that defend resources against nongroup members. There may be more or different predators at a release site not known to the individuals. Release can result in the introduction of disease and may alter the genetic/ecological integrity of the population at the release site. Release might be prohibited by federal or state laws or by permit terms. If release is required by regulation or by research design, animals should undergo a quarantine period to minimize the potential for disease transmission to resident wildlife and to safeguard the released individuals from acquiring new diseases. When group reintroductions or translocations are anticipated, feasibility studies or a formal risk analysis in advance of the release might be advisable. Federal and state/provincial agencies should be contacted well in advance to obtain authorization (including permits) for the proposed release of nonindigenous animals into the wild.

We wish to reiterate that the release of animals held in captivity should be evaluated on a case-by-case basis depending on species and environment. Taxon guidelines and consultation with other researchers can provide valuable insights for IACUC deliberations.

Other options include maintaining animals in captivity, donating animals to zoos or other organizations, or euthanasia and donation of specimens to museums and teaching collections.

If animals can be maintained in captivity and are suitable for future studies, the retention of the animals can help to reduce the overall number of individuals needed. Although this principle typically refers to reduction of the number of individuals in a single study, the overall reduction in the number of animals across two or more studies is consistent with and fulfills the purpose of the reduction principle.

Assuming that they have proper permits and appropriate facilities, zoos or animal sanctuaries may be willing to accept some or all of the animals. "Rescue" operations that find homes for animals that are suitable as pets are another option, but it can be difficult to find a reputable organization. Ethical concerns arise when donating animals to facilities that will breed animals or give or sell surplus animals to others who will breed them or sell them for "canned hunting."

If an animal must be euthanized, plans should be made to donate the specimen to a museum or a teaching collection. If no museum or teaching collection will accept the specimen, the carcass should be disposed of in accordance with applicable regulations. Animals that have been treated with drugs or that tested positive for transmissible disease should not be disposed of in the wild; incineration is the best option.

Does the AWA Apply to Federal Agencies?

AWA

Under 7 USC 2143(c), Standards and certification process for humane handling, care, treatment, the answer is clearly yes:

> In the case of Federal research facilities, a Federal Committee shall be established and shall have the same composition and responsibilities provided in subsection (b) of this section, except that the Federal Committee shall report deficiencies or deviations to the head of the Federal agency conducting the research rather than to the Animal and Plant Health Inspection Service. The head of the Federal agency conducting the research shall
>
> be responsible for—
>
> (1) all corrective action to be taken at the facility; and
>
> (2) the granting of all exceptions to inspection protocol.

Under the definition section [7 USC 2132(o)], "[t]he term "Federal research facility" means each department, agency, or instrumentality of the United States which uses live animals for research or experimentation." The terms "research" and "experimentation" are not defined.

PHS Policy

Under the Health Research Extension Act of 1985 [42 USC 289(d)] which amended the Public Health Service Act the law applies only to research funded by the Public Health Service. Therefore, PHS Policy does not apply unless the research has been funded by the PHS.

USGP

"Whenever US Government agencies develop requirements for testing, research, or training procedures involving the use of vertebrate animals, the following principles shall be considered; and whenever these agencies actually perform or sponsor such procedures, the responsible Institutional Official shall ensure that these principles are adhered to" (IRAC 1985).

Discussion

There is no doubt that the AWA applies to federal agencies, and the requirements are the same as for nonfederal institutions, with the exception of the reporting requirement, which directs agencies to report deficiencies and deviations to the head of the agency rather than to APHIS.

Some agencies also issue research permits, and in some instances, those permits are subject to review by the agency IACUC in addition to the review by the PI's institution's IACUC.

The National Park Service (NPS) IACUC reviews projects undertaken by NPS staff. Permits for the use of vertebrate animals in NPS units for research, teaching, or exhibition (where those projects do not fall under the field study exemption), the permit applicant must have IACUC approval before issuance of a permit from the hosting NPS unit. In addition, the applicant must either submit the protocol to the NPS IACUC for approval or for NPS IACUC concurrence with the approval of the primary IACUC.

The NPS unit will refrain from issuing the permit until the applicant submits an IACUC approval either from the NPS IACUC or another IACUC together with a statement of concurrence from the NPS IACUC stating that the NPS IACUC agrees with the primary IACUC's decision.

Under Policy Directives 04-112 and Procedural Directive 04-012-01 (NOAA 2015), the National Oceanic and Atmospheric Association's National Marine Fisheries Service (NMFS) created three regional IACUCs; each covers two of the NMFS Science Centers: Northeast/Southeast Science Centers, Southwest/Pacific Islands Science Centers, and Northwest/Alaska Science Centers. Any work conducted under a permit held by NMFS or conducted by a NMFS PI or a non-NMFS PI must be reviewed by one of these IACUCs. If the permit is held by a non-NMFS investigator, an IACUC approval from the PI's institution is required.

The USDA National Wildlife Research Center (NWRC) is the federal institution devoted to resolving problems caused by the interaction of wild animals and society. The center applies scientific expertise to the development of practical methods to resolve these problems and to maintain the quality of the environments shared with wildlife. Every research project conducted by NWRC researchers must be submitted to the agency's IACUC for review and approval. The NWRC does not issue permits.

Each US Geological Survey (USGS) Biological Resource Discipline Science Center has an IACUC. The USGS does not issue permits other than those issued by the USGS Bird Banding Laboratory, which does not require IACUC approval. Many, if not most, banding permittees are not subject to the AWA or PHS Policy in that they are not receiving federal funding and are not purchasing or transporting live animals in commerce. The general regulatory provision pertaining to all wildlife permits issued by USFWS agencies requires that "[a]ny live wildlife possessed under a permit must be maintained under humane and healthful conditions" (50 CFR 13.41).

Some agencies are grappling with the applicability of the AWA to activities characterized as management rather than research. Many of these activities, however, can be described as applied research if the purpose of the activity is to inform management decisions. As noted above, neither the AWA nor the USGP defines the terms "research" or "experimentation." In some cases the distinction seems clear. Some federal agencies, such as USDA Wildlife Services, have both research functions (the NWRC) and operational functions that involve the deliberate reduction in the number of animals to reduce predation on livestock, control or eliminate invasive species, control the spread of wildlife disease, or otherwise reduce conflict between humans and other animals. The operational functions might entail poisoning of flocks of crows or starlings or the killing of predators or other nuisance animals. It would be difficult to characterize these operations as research. By contrast, the US Fish and Wildlife Service engages in many activities that are intended to assess the conditions of wildlife populations or assess the outcome of particular management actions. The latter activity has come to be known as adaptive resource management. In that it entails the deliberate manipulation of wildlife or their habitats, includes a planned assessment phase, and entails the testing of competing hypotheses or determining the likelihood of a particular outcome, adaptive resource management could be described as experimentation to achieve a specific desired management outcome. The fieldwork methods used do not differ from those of basic research; common methods involve capture and marking, population estimates made on foot (American Woodcock Singing-Ground Survey), aircraft overflight (seaduck, ungulate surveys), habitat manipulation (Kirtland's warbler), and elimination of competitive species including both native competitors (brown-headed cowbirds to protect Kirtland's warbler) and non-native species (feral pigs to protect native Hawai'ian birds).

The Forest Service (FS) conducts both research and management. Its Wildlife Program manages wildlife (e.g., moving wild sheep to prevent disease transmission from domestic sheep; burning to maintain habitat for grassland species; removal of feral horses and burros on National Forest System lands) as well as a wildlife research program. The FS Research and Development program conducts research independently and collaboratively with university partners. As of late 2015, the FS has initiated an IACUC process to review the work conducted by the Research and Development program. Much like the NPS, the FS IACUC will not necessarily require submission of protocols for collaborative work if the protocol has been approved by the IACUC for the research partner, but the FS IACUC will review the primary IACUC's decision and will allow the research to proceed if it concurs with that decision. In addition, the FS IACUC will serve in an advisory capacity for the FS Wildlife Program's management programs.

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